Evolution of synchronization protocols and use of fixed time artificial insemination in beef cattle in South America

Gabriel Bó,[1] Pietro Baruselli,[2] Alejo Menchaca,[3] Reuben Mapletoft[4]
[1]Instituto de Reproducción Animal Córdoba and Universidad Nacional de Villa Maria Cordoba, Argentina
[2]Department of Animal Reproduction, University of São Paulo, São Paulo, Brazil
[3]Instituto de Reproducción Animal Uruguay Fundación IRAUy, Montevideo, Uruguay
[4]WCVM, University of Saskatchewan, Saskatoon, SK, Canada

Abstract

As our understanding of ovarian function in cattle improved, our ability to control it also increased. Although prostaglandin $F_{2α}$ ($PGF_{2α}$) has been used to synchronize estrus for several years, development of fixed time artificial insemination (FTAI) protocols permitted widespread application of AI for breeding management and genetic improvement in beef herds in South America. Over the last 20 years, a 10 fold increase in AI was largely due to use of FTAI. Main reasons for this growth were the possibility of obtaining acceptable pregnancy rates without estrus detection and induction of cyclicity in lactating cows that were anestrus and heifers that were prepubertal at the beginning of the breeding season. Most FTAI treatments for beef cattle in South America are based on use of progesterone (P_4) releasing devices and estradiol to synchronize follicle wave emergence and ovulation, with pregnancies per AI (P/AI) ranging from 40 - 60%. These protocols are adapted for use with sexed semen with acceptable P/AI and several protocols have been developed for re-synchronization of ovulation for a second FTAI in nonpregnant animals. Most of these protocols are implemented on a regular basis, allowing producers to access high quality genetics through bull selection and to increase overall pregnancy rates during a breeding season. Furthermore, it provided practitioners involved in those programs a new source of income and diversification of their practices to activities other than regular clinical work.

Keywords: Estrus synchronization, ovulation, fixed time artificial insemination, beef cattle, pregnancy rates

Introduction

Increasing knowledge of ovarian physiology in cattle, mainly through application of real time ultrasonography in the late 1980's, provided approaches for effective manipulation of ovarian function. Protocols designed to control both luteal and follicular function permitted synchronization of ovulation and efficient implementation of fixed time artificial insemination (FTAI).[1] Bovine practitioners around the world are now using these protocols, especially in South America.[2] Over the last 20 years, use of artificial insemination (AI) in South America increased 10 fold, due largely to the use of FTAI.[2] This presentation will briefly review the FTAI protocols currently used and describe evolution of this technology in South America.

Synchronization of ovulation and fixed time artificial insemination protocols in beef cattle

Estradiol/progesterone based treatments for fixed time artificial insemination

Estradiol and progesterone (P_4) treatments involve insertion of a P_4 releasing device and administration of 2 mg of estradiol benzoate (EB) on random days of the cycle (day 0; to induce follicle atresia and synchronize follicular wave emergence 4 days later), $PGF_{2α}$ at the time of P_4 device removal on days 7, 8 or 9 (to ensure luteolysis) and the subsequent application of 1 mg EB 24 hours later, GnRH or pLH 54 hours later, or 0.5 or 1 mg of estradiol cypionate (ECP) at the time of P_4 device removal to synchronize ovulation.[1] Another treatment instrumental in successful application of FTAI in South America is administration of equine chorionic gonadotropin (eCG) at the time of removal the P_4 device,[1,3] as this improves growth of dominant follicle and increases ovulation rate[4,5] and circulating P_4 concentrations in the subsequent luteal phase in cows with postpartum anestrus.[5,6] In South America, 80% of suckled cows and 50% of yearling heifers are not cycling at the beginning of breeding season in most commercial herds.[7] Therefore, use of eCG has permitted increases in P/AI from 30 - 35% to 45 - 60% in *Bos indicus*[1,4] and *Bos taurus* herds[5,7] with high incidences of postpartum anestrus.

GnRH based treatments

GnRH based protocols are used widely for beef cattle in North America and Europe. Although GnRH use is limited in South America because of cost and availability of less expensive estradiol esters, there is increased interest in GnRH based protocols among practitioners, due to political pressure from the European Union to avoid estradiol treatments in EU certified herds. Most commonly used GnRH protocol is called CoSynch; GnRH is administered at the time of FTAI to synchronize ovulation.[8] In general, CoSynch protocols have included insertion of a P_4 device to overcome poor ovulation rates after first GnRH in heifers[9] and in anestrus postpartum suckled beef cows.[10] Data on the addition of eCG to GnRH/P_4 based treatment protocols are more controversial, with reported improvements in P/AI in *Bos indicus*[11] and *Bos taurus* cows in postpartum anestrus[12] and in primiparous *Bos taurus* cows that were not presynchronized.[13] However, no improvement in P/AI has been reported in *Bos taurus* cows with low incidences of postpartum anestrus and moderate to high body condition scores (BCS).[14]

Extending the proestrus period in FTAI protocols

Protocols for FTAI that prolong the period from P_4 device removal to ovulation were developed with the objective of lengthening the interval of preovulatory estradiol exposure and improving uterine function and early embryo development.[15,16] This protocol is referred to as the 5 day CoSynch + P_4 and reported to result in higher P/AI than 7 day CoSynch + P_4 in beef cows.[15,17] Main changes in this protocol are a reduced interval of presence of P_4 device from 7 to 5 days, to avoid adverse effects of persistent follicles on fertility in cows not ovulating to first GnRH, and a prolonged interval from P_4 device removal to GnRH treatment to lengthen proestrus and increase exposure to elevated circulating estradiol concentrations prior to ovulation.[18] Higher estradiol concentrations in the preovulatory period have been associated with an increased ability of the uterus to support conceptus development[19,20] and lower embryonic losses in the interval between maternal recognition of pregnancy and placental attachment.[21]

Because of the shorter interval between the first GnRH and induction of luteolysis in the 5 day CoSynch + P_4 protocol, a single treatment of $PGF_{2\alpha}$ was not effective in inducing luteolysis in beef cows that ovulated in response to GnRH;[22] 2 doses of $PGF_{2\alpha}$ 8 -12 hours apart were required for high P/AI.[23] In a large field trial with 2465 postpartum beef cows, P/AI was greater ($p < 0.05$) in cows receiving two $PGF_{2\alpha}$ treatments, 8 hours apart (55%) than those receiving only one $PGF_{2\alpha}$ (48%), with those receiving two $PGF_{2\alpha}$ at the same time being intermediate (51%).[16] Hence, double $PGF_{2\alpha}$ given 8 - 24 hours apart seemed necessary to maximize fertility with the 5 day protocol. If farm conditions do not permit the extra handling, a double dose of $PGF_{2\alpha}$ given at device removal would be an acceptable alternative, or the first GnRH may not be administered, especially in heifers (see below).

Use of the 5 day CoSynch + P_4 protocol has also been investigated in *Bos indicus* breeds in South America, with lower P/AI in suckled Nelore cows than with the conventional 8 day estradiol/P_4 based protocol[24]. However, an important difference in this study was that 400 IU eCG was used in the estradiol/P_4 based protocol but it was not used in the 5 day CoSynch + P_4 protocol. To confirm the importance of eCG, we found no differences in P/AI in cycling cows treated with either 5 day CoSynch + P_4 or estradiol/P_4 based protocol without the use of eCG, but P/AI was higher in cows in postpartum anestrus that received 400 IU eCG at P_4 device removal (5 day CoSynch + P_4: 46.3%, 120/259; estradiol/P_4 based: 54.5%, 151/277) than in cows treated with 5 day CoSynch + P_4 but without eCG (26.8%, 71/265; $p < 0.05$).[12]

The 5 day CoSynch + P_4 protocol was examined in heifers,[25] with some modifications; for example, Colazo and Ambrose[26] and Cruppe et al.[27] reported that P/AI did not differ in heifers that did not receive GnRH at the time of insertion of a P_4 device. The important issue with this approach is that heifers are not highly responsive to GnRH administered at random stages of the follicular wave and the 5 days of P_4 are not sufficient for development of a persistent follicle; by not administering the first GnRH, a single injection of $PGF_{2\alpha}$ is all that is required to induce luteal regression.

Controversy still exists concerning the necessity of using one or two doses of $PGF_{2\alpha}$, when the first GnRH is administered in the 5 day protocol, with no reported differences[28] and higher pregnancy rates when two doses of $PGF_{2\alpha}$ were used, with intervals ranging from 6 - 24 hours.[25,29] Optimal timing of FTAI remains controversial; Kasimanickam et al.[28] reported higher P/AI in heifers inseminated at 56 hours after P_4 device removal than at 72 hours, whereas Day[25] suggested FTAI 60 - 66 hours after P_4 device removal or insemination 12 hours after estrus observed using tail patches or tail paint and FTAI/GnRH to all those not in estrus by 72 hours. Expression of estrus influences P/AI in cows[30] and Colazo et al.[31] have reported similar findings in heifers inseminated with sexed semen, suggesting the possibility of splitting insemination times based on expression of estrus (i.e. delaying insemination in animals not detected in estrus by 72 hours after removal of a P_4 device).

Estradiol/P_4 based protocols with extended proestrus

We recently conducted a series of experiments to evaluate an estradiol/P_4 based protocol with a prolonged proestrus interval, which has been named JSynch.[32] The treatment consists of administration of 2 mg EB at the time of insertion of a P_4 device that is removed 6 days later. A single dose of $PGF_{2\alpha}$ and sometimes 300 (heifers) or 400 (cows) IU of eCG are given at device removal. All cattle receive GnRH and are FTAI, without estrus detection, 72 hours later (day 9). If estrus detection aids, such as tail paint or patches are used, the time of AI in heifers in estrus can be advanced to 60 or 66 hours after P_4 device removal and only those not displaying estrus by 72 hours receive GnRH and FTAI at that time.[33]

Studies using real time ultrasonography and hormone determinations have shown that the growth rate of the ovulatory follicle and serum P_4 concentrations in the ensuing luteal phase were greater in heifers treated with the 6-day JSynch protocol than in those treated with the conventional 7 day estradiol/P_4 based protocol. Furthermore, immunohistochemistry and real-time PCR of biopsies taken on day 6 after ovulation suggested that heifers treated with the prolonged proestrus protocol had a more mature uterine environment for embryo development.[34] In most studies conducted in beef *Bos taurus* heifers, P/AI was higher with the JSynch protocol than with the conventional estradiol/P4 protocol (Table 1).[33,35] Although P/AI did not differ in a study in *Bos indicus* heifers,[36] P/AI was higher in Nelore heifers treated with the JSynch protocol than those treated with the conventional protocol in a more recent study (Table 1).[37]

Table 1. Pregnancy rates in beef heifers treated with the JSynch or with the conventional estradiol based treatment protocols.

Experiments - Reference	JSynch	Conventional	p value
1 - Bó et al. 2016 (n = 583)	59.7% (175/293)	53.1% (154/290)	0.12
2 - Bó et al. 2016 (n = 208)	67.9% (70/103)	46.6% (49/105)	0.01
3 - Bó et al. 2016 (n = 555)	37.8% (104/275)	49.3% (138/280)	0.01
4 - Bó et al. 2016 (n = 2.349)	56.1% (631/1,125)	50.7% (620/1,224)	0.01
5 - Motta et al. 2016 (n = 785)	52.9% (207/391)	53.6% (211/394)	0.8
6 - Pincinato et al. 2018 (241)	62.9% (68/108)	48.1% (64/133)	0.01
Total	54.7% (1,255/2,295)	50.1% (1,236/2,426)	0.01

In Experiments 1, 2 and 3, heifers did not receive eCG at device removal.
In Experiments 4, 5 and 6, all heifers received 200 (Experiment 4) or 300 IU eCG at P_4 device removal.
In Experiment 2, heifers were in low body condition score (4 - 6 in a 1 - 9 scale) and losing weight, whereas in all other studies, heifers were in good body condition score (6 - 7) and gaining weight.

Fixed time artificial insemination using sexed semen

Improvements in sorting and freezing procedures with sexed semen and increasing number of sperm from 2.1 x 10^6 - 4 x 10^6 sperm per straw has created interest in the application of this technology in beef herds. The new product is called SexedULTRA™.[38] Experiments with SexedULTRA™ semen have been performed in beef heifers and suckled cows in South America. The overall recommendation with the conventional estradiol/P4 based protocols is to delay insemination time with sexed semen in both heifers and cows by 12 hours and to inseminate only those that have shown estrus by the time of FTAI.[33,39]

We performed 2 studies to evaluate P/AI in beef heifers (n = 850) and suckled cows (n = 877) treated with the JSynch protocol and inseminated with SexedULTRA™ or nonsorted (conventional) semen from split ejaculates of the same Angus sires.[33,40] All cattle were treated with the JSynch protocol as described above and were tail painted for estrus determination. Cattle with the paint rubbed off by 60 or 72 hours after device removal were inseminated at 72 hours, whereas those not displaying estrus by 72 hours received GnRH at that time and were inseminated at 84 hours. In heifers, the overall P/AI were 49.3% for those inseminated with SexedULTRA™ and 58.3% for those inseminated with conventional semen ($p < 0.05$, Table 2).[33] In suckled cows, the overall P/AI was 45.3% for those receiving SexedULTRA™ semen and 68.3% for those inseminated with conventional semen ($p < 0.05$, Table 2).[40] In summary, protocols designed for FTAI in cows and heifers can be adapted for sexed semen. Although, P/AI are lower than those obtained with conventional semen, delaying the time of AI or limiting AI to those showing estrus would result in P/AI ranging from 40 and 50% and often higher.

Table 2. Pregnancy rates in Angus heifers and suckled cows inseminated with sexed (SexedULTRA™) or conventional semen according to the expression of estrus (tail paint reading) and time of insemination (AI) after removal of the progesterone releasing device.

	n	Estrus 60 hours AI 72 hours	Estrus 72 hours AI 72 hours	No estrus at 60 or 72 hours AI 84 hours	Total
Beef heifers					
SexedULTRA (4 x 10^6 sperm)	426	59.0% (104/176)	45.5%[a] (61/134)	38.8% (45/116)	49.3%[a] (210/426)
Conventional (25 x 10^6 sperm)	424	69.2% (119/172)	58.0%[b] (79/136)	42.2% (49/116)	58.3%[b] (247/424)
Beef suckled cows					
SexedULTRA (4 x10^6 sperm)	435	40.8%[a] (93/228)	43.0%[a] (34/79)	56.5%[a] (70/124)	45.3%[a] (197/435)
Conventional (25 x 10^6 sperm)	442	65.8%[b] (154/234)	67.9%[b] (57/84)	74.2%[b] (92/124)	68.6%[b] (303/442)

[ab] Denotes differences in P/AI between sexed or conventional semen ($p < 0.05$).

Resynchronization treatments

With P/AI ranging from 40 - 60% after the first AI, producers are asking researchers and practitioners for development of protocols that would achieve higher P/AI in a breeding season or even the possibility of developing protocols in which 85 - 95% of the cows get pregnant only by AI, without the necessity of cleanup bulls. Several resynchronization protocols were investigated over the years; however, most require estrus observations or an interval of approximately 40 days between the first and second FTAI.[35,41] In order to be able to inseminate nonpregnant cows as early as possible, resynchronization treatments must start earlier than pregnancy diagnosis. One protocol developed involves insertion of a used P_4 device (i.e. previously used in the first synchronization) on days 14 - 16 after the first AI and its removal on day 23 when GnRH is administered. Ultrasonographic pregnancy diagnosis is performed on day

30 and those that are non-pregnant receive $PGF_{2\alpha}$ combined with either 0.5 mg ECP at that time or GnRH at the time of FTAI on day 32. P/AI for the first and second FTAI at an interval of 32 days and the overall P/AI in a field trial involving 6431 beef cows and heifers were 57, 51 and 79%, respectively.[35]

Two other protocols for resynchronization are called Resynch 22 and Resynch 14.[41] In the Resynch 22, cows receive 2 mg EB and heifers 1 mg EB at the time of P_4 device insertion on day 22 after FTAI. Pregnancy diagnosis is performed at device removal on day 30 when nonpregnant cattle receive $PGF_{2\alpha}$ and ECP and are inseminated on day 32. In a recent field trial, the use of three consecutive FTAI with the Resynch 22 protocol had a similar overall pregnancy rate (87.8%, 663/755) as achieved using cleanup bulls after two FTAI using Resynch 22 (87.7%, 263/300) and greater pregnancy rate than one FTAI followed by bull exposure (77.1%, 347/450).[42] In Resynch 14, the initial treatment starts 14 days after FTAI with reinsertion of a used P_4 device and administration of 100 mg P_4 IM.[41] Pregnancy assessment is done by the evaluation of the vascularity of the CL using Doppler ultrasonography at the time of P_4 device removal on day 22. Nonpregnant cattle receive $PGF_{2\alpha}$ and ECP at that time and are inseminated on day 24. In a recent study, similar P/AI were observed for Resynch 22 and Resynch 14 groups following the first FTAI (48% versus 53%; $p = 0.57$) and resynchronization (56 versus 51%; $p = 0.37$), respectively. However, Resynch 14 reduced the interval between FTAI, which resulted in a 21-day pregnancy rate of 87.5% compared to 66% with Resynch 22.[43] In conclusion, with the existing resynchronization programs it is now possible to breed beef cows exclusively with FTAI, eliminating the need for estrus detection and cleanup bulls.

Application of fixed time artificial insemination in South America

South American countries represent the largest beef herd in the world. In 2016, Latin American countries had approximately 359 M cattle (accounting for 24% of the world's cattle population) and produced 15.1 M tons of beef.[2] Of the main beef producing countries in South America, Brazil (60.8% of the total), Argentina (14.7%) and Uruguay (3.3%) are also prominent in development and application of assisted reproductive biotechnologies. Through training and continuing education programs, many of which one or more of the authors have organized or participated in, these technologies are being used increasingly in other South American countries. It is through these efforts, in partnership with the pharmaceutical industry, that the ruminant reproductive revolution in South America has become widespread.

Brazil and Argentina, in particular, are not only very active in research but also in continuing education and postgraduate student training in reproductive biotechnology. In just one program, the Specialization in Bovine Reproduction at the Institute of Animal Reproduction in Cordoba Argentina (IRAC), more than 1200 practitioners from most South American countries have taken postgraduate training since 2002. Most beef herds in Brazil and Paraguay are composed of *Bos indicus or Bos indicus* crosses, whereas Argentina and Uruguay often have more *Bos taurus* breeds. *Bos indicus*-influenced synthetic breeds have increased in Argentina due to the recent expansion of agriculture that has pushed cattle breeding to less favorable environments. It is noteworthy that *Bos indicus* breeds tend to have prolonged postpartum anestrus and low body condition scores on pasture, with an increased interval from calving to conception and low pregnancy rates.[45] However, in a study involving 21,329 suckled *Bos taurus* cows and heifers examined by ultrasonography at the beginning of the breeding season in commercial beef farms in Uruguay, 80 - 90% of cows and 40% of heifers are not cycling (i.e. without a CL) at the beginning of the breeding season.[7] Thus, in these pasture-based cow calf production systems, synchronization protocols are not only necessary to produce pregnancies by AI during a short breeding season, but also to induce cyclicity in cows in anestrus that would likely not get pregnant if not treated.[46] These are the main reasons why FTAI has been incorporated into the breeding schemes of most progressive producers.[1,35] Breeding objectives have been to inseminate early in the breeding season, early ultrasonographic pregnancy diagnosis and re-insemination of nonpregnant cattle as soon as possible.[41]

As was mentioned previously, because estradiol preparations have been available in South America, most FTAI protocols include estradiol as a means of synchronizing follicle wave emergence and ovulation.[1] As herd cyclicity and body condition scores are usually low, progestin devices and eCG are usually included in the synchronization protocols.[1,3,6,35] Protocols of 7, 8 or 9 days progestin treatment have resulted in similar pregnancy rates, and shortened progestin protocols with a lengthened proestrus involving either estradiol or GnRH have been used with success.[41]

FTAI is increasing the use of AI, especially with *Bos taurus* sires on *Bos indicus* cows in Brazil, Paraguay and the northern part of Argentina. In Brazil, the use of FTAI increased from ~ 1 M protocols in 2005 (11% of all AI) to 10.5 M protocols in 2015 (77% of all AI), and a further increase to > 11 M FTAI in 2016 (Figure 1).[2] This represents more than a 10 fold increase in the use of AI in the Brazilian beef herd. In Argentina, there were < 200 x 10^3 FTAI protocols in 2002 and this has increased to ~ 2.5 M in 2015 and > 3 M in the last breeding season. This accounts for > 90% of inseminations done in beef cattle in Argentina, which is about 15% of the beef breeding females in the country (Figure 1). Uruguay and Paraguay have experienced similar trends, with 300 x 10^3 FTAI in Uruguay and 500 x 10^3 FTAI in Paraguay, representing ~ 10% of their breeding females. In total, > 15 M cattle were inseminated by FTAI in these four countries over the past year. The adoption of this technology has been suggested to be an excellent example of a technological change in the production sector emerging from scientific developments in the academic sector.[2]

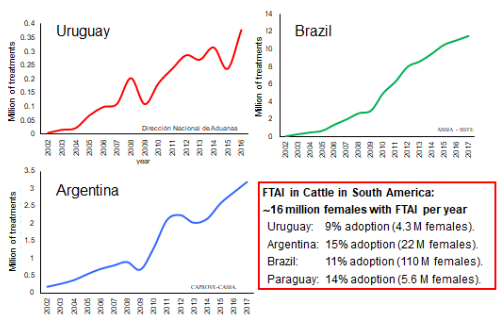

Figure 1. Evolution of the application of FTAI in Uruguay, Brazil and Argentina. Data were calculated based on official semen and hormone sales in the three countries. Although the evolution figures for Paraguay are not available, the number of cows FTAI in the 2017 breeding season is included for reference and comparison.

Impacts of use of fixed time artificial insemination in commercial beef herds

One of the main advantages of implementing FTAI programs in a beef herd is that more cows can be impregnated earlier in the breeding season to genetically improved bulls, resulting in heavier weaning weights (reviewed[46]). Between 40 and 60% of the cows could become pregnant on the first day of the breeding season, resulting in a higher number of cows calving at the beginning of the calving season. Their calves will be older and heavier at weaning, and the use of genetically improved bulls will also result in heavier calves at weaning.

Impact of FTAI is equally effective in various management systems in Argentina and Brazil.[45,46] A recent study[47] compared weaning weights and kilograms of beef per cow using estradiol/P_4 based protocols with FTAI or bull exposure versus traditional natural service. At the beginning of the breeding season, cows were randomly allocated to three treatment groups. Cows in two groups received the conventional estradiol/P_4 based treatment with eCG at device removal and then half of those cows were FTAI and the other half directly exposed to 5% bulls for 90 days immediately after P_4 device removal. Cows in the control group were exposed directly to 5% bulls without synchronization treatments at the same time that devices were removed in two synchronized groups. Cows in FTAI group were also exposed to 5% bulls 10 days after FTAI for the remainder of the breeding season. Pregnancy diagnosis was performed by four sequential ultrasonographic examinations every 30 days. Pregnancy rates after the first 25 days and at the end of the breeding season were significantly higher in cows in the two synchronized groups than in those in natural service ($p < 0.05$; Table 3). However, the most important finding was the difference in weaning weights. Calves in FTAI group were 27.6 kg heavier and those in the synchronized group that were directly exposed to bulls were 19.1 kg heavier than those in the natural service group. Furthermore, cows in the FTAI group produced 69 kg more of beef at weaning than those exposed to natural service (Table 3). Changes in calving patterns and weaning weights have been considered the main reason why the most progressive cow/calf producers in South America have adopted this technology to improve profits.[2,45,46]

Table 3. Pregnancy rates during the first 25 days and at the end of a 90 day breeding season and weaning weights of calves produced, in beef cows that were synchronized with an estradiol/P_4 protocol and exposed to bulls (Synch with bulls), or FTAI and then exposed to bulls for the remainder of the breeding season (FTAI plus bulls), or directly exposed to bulls without any synchronization treatment (natural service).

	25 day Pregnancy rate (%)	Overall Pregnancy rate (%)	Calf weaning weights (kg)
Synch with bulls	54.1% (60/111)^{b}	94.6% (105/111)^{b}	204.8 ± 28.8^{b}
FTAI plus bulls	58.1% (61/105)^{b}	96.2% (101/105)^{b}	216.8 ± 19.8^{c}
Natural Service	25.5% (35/137)^{a}	83.9% (115/137)^{a}	191.2 ± 18.2^{a}

^{abc} Denotes differences within pregnancy rates or weaning weights ($p < 0.05$).

Conclusion

Ability to control follicular wave emergence and ovulation in cycling and noncycling cattle has allowed successful implementation of FTAI in beef herds in South America. Treatments with GnRH or estradiol and P_4 releasing devices made FTAI possible in beef cattle. These treatments are practical and easy to perform by the farm staff and are efficient and useful for inducing cyclicity at the beginning of the breeding season. Practitioners in most South American countries are using these protocols, which has facilitated the use of AI in beef herds and application of other assisted reproductive technologies. Currently, FTAI procedures account for > 85% of all AI done in beef cattle in Argentina, Brazil, Uruguay, and Paraguay. Successful application of FTAI in South America is resulting in the dissemination of new and improved genetics and increased reproductive performance in all classes of cattle, with a corresponding increase in economic activity.

Conflict of interest

There are no conflicts of interest to declare.

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