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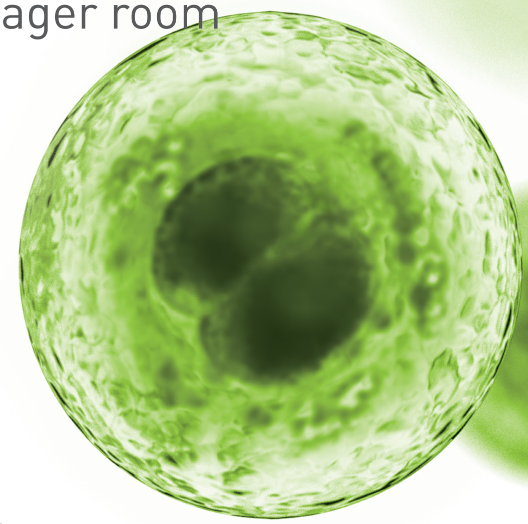
PRECISION BREEDING: PRACTICAL ASSISTED REPRODUCTION FOR BEEF PROFITABILITY

Repro360 Symposium

Beef Australia 2018

May 8th 2018 in the Gallagher Livestock Manager room



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Dr Gabriel Bó

Dr Enoch Bergman

Darren Hamblin

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Utilising Reproductive Technologies for Carcass Value

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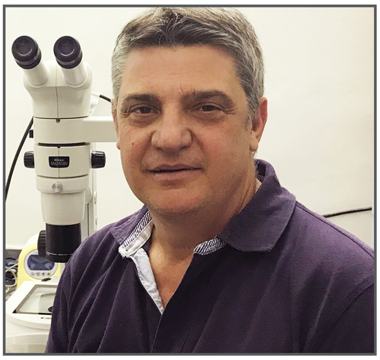

Dr. Gabriel Bó, is currently President and Director of Research and Post-graduate training of the Instituto de Reproducción Animal Córdoba (IRAC) and Professor of Obstetrics and Biotechnology of Reproduction at the Veterinary School of the Instituto de Ciencias Basicas y Aplicadas, Universidad Nacional de Villa Maria in Cordoba, Argentina. Dr. Bó has been working for many years on applied research and the commercial application of fixed-time Artificial Insemination and Bovine Embryo Technologies. He is Past-President of the International Embryo Transfer Society (IETS) and founding member of the Argentine Embryo Technology Society (SATE). Dr. Bó has lectured in courses on advanced reproductive technologies and has been invited speaker in the main conferences around the world. During his career Dr. Bó has obtained several awards like the “Taurus Award” in Argentina in 2008, the IETS Distinguished Service Award in 2015, the “Asis Roberto de Bem” of the SBTE in 2015 and he has recently being named Member of the National Academy of Agricultural and Veterinary Sciences of Argentina. Finally, he has published more than 100 manuscripts in referred journals and book chapters, more than 150 invited reviews in conference proceedings around the world and more than 450 abstracts.

Dr Enoch Bergman

Dr Enoch Bergman is a beef cattle veterinarian, consultant, and partner within Swans Veterinary Services, located in Esperance, Western Australia. He travels widely across the state providing veterinary services and advice to southern beef producers, seed stock producers, lot feeders, and pastoralists. Further, he regularly travels throughout Australia speaking to other veterinarians or producers on a range of topics relating to improving reproductive efficiency or integrating breeding technology into commercial herds. He was president of the Australian Cattle Veterinarians from 2014 to 2016, a special interest group of the Australian Veterinary Association. During his tenure he worked hard to strengthen his association’s relationships with other peak industry bodies. He is a passionate advocate for the Australian beef sector and a vocal proponent of the veterinary industry’s role in supporting it.

Darren Hamblin

The Hamblin family run their 6,000 Wagyu herd over 46,000 acres in central Queensland and the Darling Downs. They began breeding Wagyu in 2000 by artificially inseminating their Brahman and Brangus cows. They have since purchased higher-content Wagyu crossbred cows to boost breeder numbers. The Hamblins' Wagyu enterprise also trades under the Strathdale Wagyu and Masterbeef brands. The Hamblins' breed Fullblood, Poll Purebred and crossbred Wagyu cattle. Their herd is raised on buffel grass pastures in central Queensland. They are lot fed in Southern Queensland and marketed as carcase sales in Queensland and New South Wales.

The Hamblins used shorthorn as a base breed in order to source the large number of cows needed for a sizeable land addition in 2010. Their offspring have been the nucleus for their progeny test selection process for high-performance sires. The enterprise has largely used artificial insemination, but in 2014 the Hamblins also focused on IVF to increase their herd's volume of high-performing genetics from superior sires and dams. Their main practice has since been a combination of IVF & MOET flush programs: their Wagyu Cows are recipients of over 1,000 Wagyu embryos a year.

Hamblin Pty Ltd won gold medals in the Australian Wagyu Association's Branded Beef Competition in 2014, 2015 and 2016. Hamblin entries also placed 1st, 2nd, 3rd, 4th & 5th in the Inaugural Ekka Wagyu Paddock to Plate competition in 2017. They also won five out of six categories with an F1 animal in the 2010 Pacific Carcass Competition.

Precision Breeding: How to Get the Best Results Using Fixed-Time AI

Dr Gabriel A. Bó

Introduction

Artificial Insemination (AI) is one of the main techniques used worldwide to disseminate desirable genetics among beef and dairy herds. However, the widespread implementation of AI in beef herds is very recent and is mainly due to the use of protocols that allow the AI of large groups of animals at a given time, commonly called fixed-time artificial insemination (FTAI). In South America, the numbers of cattle involved in FTAI programs during the breeding season has increased dramatically in the last 15 years, from less than 100,000 in the early 2000s to about 3,000,000 in Argentina and about 10,000,000 in Brazil (Bó et al., 2016). The objective of this brief review is to present and describe the treatments available and discuss their impact on beef cattle fertility.

Traditional Ovulation Synchronisation Treatments in Beef Cattle

There are basically two types of FTAI protocols currently used in beef cattle; GnRH-based and oestradiol-based protocols, both of which are combined with progesterone-releasing devices. Oestradiol and progestin treatments consist of insertion of a progesterone-releasing (P4) device and the administration of 2 mg of oestradiol benzoate (ODB) on Day 0 (to induce follicle atresia and synchronise follicular wave emergence), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at the time of P4-device removal on Days 7, 8 or 9 (to ensure luteolysis) and the subsequent application of 1 mg ODB 24 hours later, or GnRH 54 h later (Bó et al., 2013). A recent analysis from 431,000 FTAI performed in Argentina reported a mean pregnancy rate per AI (P/AI) of 50%, ranging from 6% to 100%. The median P/AI was between 51 to 60% (41% of the herds) followed by 60 to 70% in 24% of the herds and 41 to 50% in 23% of the herds. The mean number of animals inseminated on a given day was 245. The mean P/AI was higher in *Bos taurus* herds (54.9%; n=68,878) than in *Bos indicus*-influenced herds (48.7%, n=95,152). Furthermore, most treatments applied to suckling beef cows in also involve the application of equine chorionic gonadotropin (eCG) at the time of removal the progesterone-releasing device, increasing pregnancy rates by about 7 to 20% in beef cows in postpartum anoestrus (Baruselli et al., 2004) and also in heifers (Bó et al., 2013).

New Protocols for FTAI

New protocols have been developed recently that reduced the period of P4-device insertion and increasing the period from device removal to ovulation (period known as proestrus). There are basically three protocols, a GnRH-based protocol called the 5-day Co-Synch (Bridges et al., 2008) and two oestradiol-based protocols (de la Mata and Bó, 2012; Edwards et al., 2014).). The importance of a prolonged proestrus derived from a series of studies suggesting that the more consistent predictor of fertility in beef cattle was the duration of proestrus, rather than follicle diameter (Bridges et al., 2010). The prolonged proestrus was also related to lower embryonic losses in the time period between maternal recognition of pregnancy and placental attachment (Madsen et al., 2015) and increased pregnancy rates compared to the traditional 7-day Co-Synch protocol (Bridges et al., 2008). The recommended protocol for the 5-day Co-Synch program is illustrated in Figure 1 (Day et al., 2015).

The J-Synch protocol is an oestradiol-based protocol, which also have a prolonged proestrus prior to ovulation. The treatment protocol consists on the application of 2 mg ODB at P4-device insertion (Day 0), $PGF_{2\alpha}$ and 300 IU eCG is given at device removal on Day 6. No oestradiol is used to induce ovulation and heifers are inseminated and receive GnRH 72 h after device removal (de la Mata and Bó, 2012; Figure 2).

In a field trial, involving Angus x Hereford heifers, higher ($P<0,05$) P/AI were obtained in heifers treated with the J-Synch protocol compared to those treated with the conventional oestradiol-based protocol (Table 1, Bó et al., 2016).

Several studies have been performed around this protocol and several options have been developed. One option is using tail-paint at P4-device removal and heifers with the tail paint rubbed off are inseminated without GnRH.

Another option is to split the inseminations. The heifers with the tail-paint rubbed off are inseminated from 66-72 h after P4-device removal and those with the tail-paint intact received GnRH and are inseminated 8-12 h later. Finally this protocol has also allowed the use of sexed semen, in which heifers with the tail-paint rubbed off by 60 or 72 h are inseminated at 72 h with sexed semen and those with the tail paint intact receive GnRH and are inseminated at 84 h after P4-device removal.

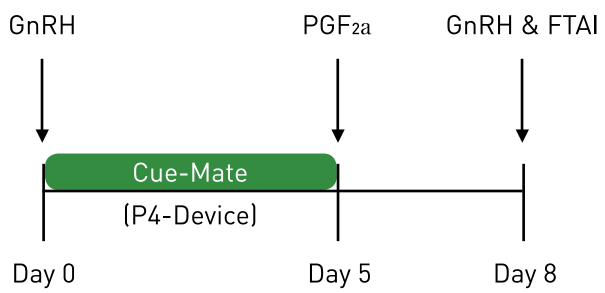

Figure 1. 5-day Co-Synch protocol in cattle. A second $PGF_{2\alpha}$ administered at the same time of the device removal or 6 to 12 h later is recommended in cows and 400 IU of eCG may also be given in cows in postpartum anestrus or poor body condition score.

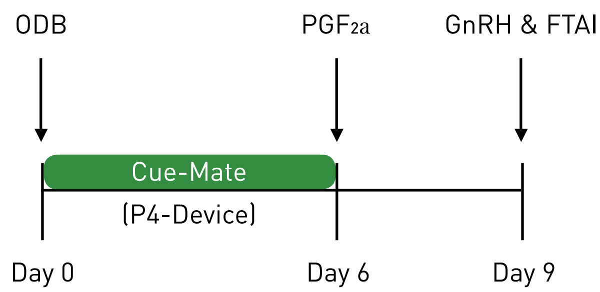

Figure 2. Recommended J-Synch protocol in cattle. The recommended interval from P4-device removal and the second GnRH and FTAI is 72 h in beef heifers. Administration of eCG (300 IU in heifers and 400 IU in cows) may also be given in those animals in anestrus, with poor body condition score or embryo recipients.

The overall P/AI involving 850 heifers inseminated using this protocol was 49.3% for sexed semen and 58.3% for non-sexed semen, with 3 out of 4 bulls exceeding 50% P/AI with sexed-semen.

possible, and 3) re-breed the non-pregnant cows as soon as possible. The easiest and most commonly used alternative to get non-pregnant cows pregnant soon after the first AI is to use clean-up bulls

Table 1. Effect of synchronisation treatment (J-Synch vs. Conventional), on P/AI in beef heifers.

	J-Synch (+ 300 IU eCG)	Conventional (+300 IU eCG)	*P*
Total (n=2,349)	**56.1% (631/1,125)**	**50.7% (620/1,224)**	**0.01**

*Heifers in the conventional treatment received 0.5 mg oestradiol cypionate at progesterone device removal (Day 7 AM) and were FTAI at 48 (Day 9 AM) or 56 h (Day 9 PM) later. In J-Synch treatment, the progesterone device was removed on the PM of Day 6 and heifers received GnRH and were FTAI 60 (Day 9 AM) or 72 (Day 9 PM) h later. (Adapted from Bó et al., 2016)

A similar approach of a prolonged proestrus and FTAI at 72 h after device removal, but by giving 1 mg EB 36 h after device removal instead of GnRH at the time of FTAI was evaluated in Brahman heifers (Edwards et al., 2014). P/AI was significantly higher in heifers treated with the prolonged proestrus protocol than those treated with the conventional 8-day protocol in one farm, but no differences were detected in two other farms. The recommended protocol is shown in Figure 3.

Re-synchronisation Treatments

Aggressive reproductive management systems comprise three strategies that can be implemented early during the breeding period: 1) inseminate all cows at the beginning of the breeding season, 2) identify non-pregnant cows as early as

for the remainder of the breeding season. However, there are other options for breeders that desire a larger percentage of AI-sired calves or wish to limit the use of bulls. Several approaches have been developed over the years; however, most require oestrus observations or an interval of

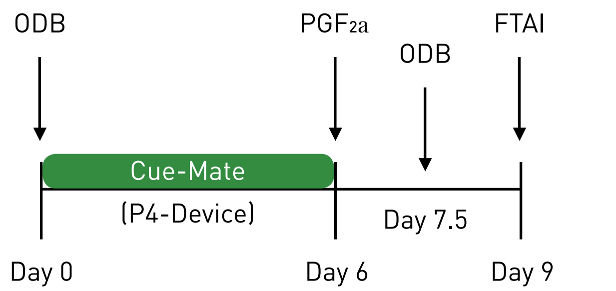

Figure 3. Short protocol for FTAI using ODB to induce ovulation. The recommended interval from P4-device removal and the second ODB is 36 h and the FTAI is performed at 72 h.

approximately 40 days between the first and second FTAI (reviewed in Bo et al., 2016). In order to be able to inseminate non-pregnant cows as early as possible, re-synchronisation treatments must start earlier than pregnancy diagnosis.

A re-synch protocol that was recently investigated is to administer 1 (heifers) or 2 (cows) mg of ODB and insertion of a P4-device on Day 22 after the first FTAI (Sa Filho et al., 2014, Pessoa et al., 2015). Pregnancy diagnosis by ultrasonography was performed at device removal on Day 30. All non-pregnant cattle received PGF2α and 0.5 mg of oestradiol cypionate at device removal and were FTAI 48 h later (Figure 4). Using this protocol the reported cumulative P/AI ranged from 68 to 75%. In a more recent field trail, the use of three consecutive FTAI with the Resynch 22 protocol had a similar overall pregnancy rate (87.8%, 663/755) as that achieved using clean-up 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; Crepaldi et al., 2017).

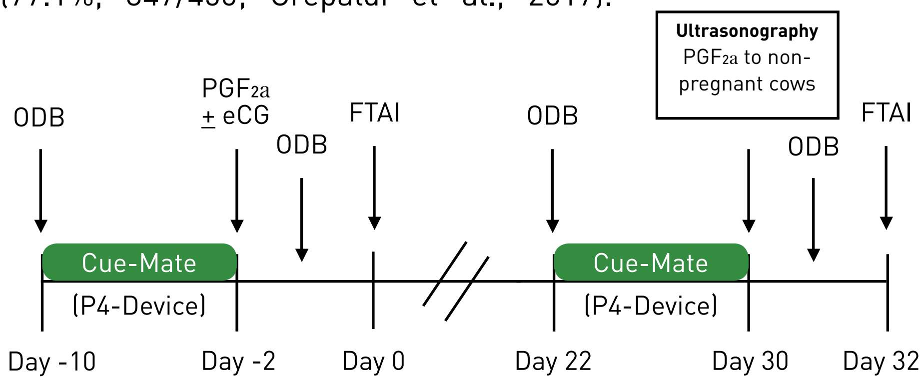

Figure 4. Re-synchronisation with unknown pregnancy status using progesterone and oestradiol-based fixed-time AI protocols in beef cattle.

Final Comments

Protocols for fixed-time AI are now an invaluable management tool to increase the number of animals bred with high-quality genetics with the extra benefits or reducing the postpartum interval in suckled cows and to compact the breeding and calving seasons in cows and heifers. The new synchronisation treatments that provide for a longer proestrus are an interesting new alternative for FTAI and have resulted in increased P/AI in beef heifers. Finally, similar approaches to those used for the first FTAI can also be combined with early pregnancy diagnosis with ultrasonography for a second or third FTAI without estrus detection resulting in similar or higher P/AI to those obtained with clean-up bulls, maximising the use of the improved genetics in a herd through AI.

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Realising the Value of the Integration of Fixed Time AI into Commercial Beef Cattle Enterprises

Dr Enoch Bergman, DVM

Introduction

Artificial Insemination is a fantastic tool to rapidly improve the genetics of Australian beef producers. Further, busy cattle veterinarians, such as myself, without significant companion animal responsibilities are often doing very little during the mating season. Prior to integrating AI into my practice, I used to tell my clients, "When the bulls are working, I'm not!"

Traditional heat detection based programs do not suit most busy beef producers. They also fail to suit most busy veterinarians! The advent of Fixed Time AI (FTAI) programs, yielding reasonable results has created fantastic opportunities for producers and their veterinarians as well!

The Primary Motivation

After delivering their first calf, heifers are under significant nutritional strain. Heifers are growing, lactating, gaining new dentition, and simultaneously trying to look after their first calf. Whilst adult cows take around 55 days to reconceive, heifers can take up to a month longer. After 280 days of pregnancy, there are only 85 days left in the year! In essence, this means that heifers that calve at the same time as the adult cow herd often fail to cycle before the bulls are put in and some might not before the bulls get pulled out! It is no wonder that the conception rates of rising three year old first calf heifers is often disappointing.

After arriving in Esperance, WA I began introducing my clients to the concept of the benefit of mating their heifers in advance of the rest of the cow herd and to shortening the mating window of their heifers as well. My rationale was to buy their heifers additional time after they calve, allowing more of them sufficient time to repair and prepare their uterus after delivering their first calf, improving the likelihood of their conceiving early in their second joining. Specifically, I advocated a six week joining, budgeting on an 80% conception rate for their heifers. I then encouraged them to preg test their heifers six weeks after the bulls finished up and to sell the empties either directly to a feedlotter or to grass finish them. Regardless, my motto was "There is no tragedy in an empty heifer." Empty heifers are worth significantly more per kilo than empty three year old animals. Further, by short and early joining them, the overall calf crop was more consistent, as the calves from heifers receive less nutritional assistance from their poorer milking mothers than their cow reared siblings.

Many producers embraced the new scheme, however, some of my clients were still a bit nervous. In order to allay their fears I set some of them up with a pre-mating synchrony program comprised of two doses of prostaglandins two weeks apart, followed by seven weeks of bulls starting at the second dose of prostaglandin. This allowed most of the heifers to cycle three times over a short period. We were able to condense the heifer calving to a seven week mating yet still achieve a more palatable 90% conception rate with this program. The caveat was that we needed a higher proportion of bulls to cover the first oestrus. We budgeted on 5% bulls for the first week of the mating program, with normal bull stocking rates thereafter.

The double prostaglandin program was good, but some producers didn't have enough appropriate bulls to cover the first round of synchrony adequately, so, I began to think about FTAI. I thought, if I could perform one round of FTAI three weeks ahead of the cow mob over the heifers, I could then plug the bulls into the heifers ten days later, leave them in for six weeks, and generally end up with approximately a six week joining. As an added bonus, I could put really safe, high genetic merit bulls over the heifers and keep the progeny!

The Secondary Motivation

When I proposed my idea, most of my clients responded that they never kept any of the progeny from their heifers. A producer's heifers should represent their best genetics! If they aren't their best genetics, by definition, that producer's herd is going backwards! I realised that they were focusing on the appearance of the heifer's calves rather than on their genetic makeup, as heifers tend to produce less milk. Further, many had traditionally been terminally crossing their heifers to inferior bulls! Many bulls purchased for use over heifers has EBV's for low birthweight BUT ALSO for low weaning weight. Some producers even terminally crossed to miniature breeds in order to manage dystocia, over their BEST GENETICS! Having a good understanding of EBV's and the value of accuracy in their determination, I sought to seek out proven calving ease bulls with great growth and maternal characteristics and to change the way my producers thought.

Putting Runs on the Board

I started fairly slowly. I ran my dream past a semen salesman acquaintance, he pulled some strings and helped me to access a $22 dollar exceptionally accurate, extremely low birth weight, calving ease bull, with incredibly short gestational length and well above average weaning weight. He was called Final Answer. I started by targeting smaller producers that had suffered through a tough calving. Many of them had threatened to stop raising their own heifers, frustrated by the unreliability of the EBV's of off the shelf unproven bulls.

Early on, I set my producers up with the following program:
Bomerol + Cue-Mate on day 0
Ovuprost, Pregnecol and pull Cue-Mate on Day 8
Bomerol and apply heat detection devices on Day 9
FTAI 28 hours later on Day 10 (52 hours after Cue-Mate removal).

Producers found the program straight forward and much less labour intensive than they expected. For myself, I simply arrived at the appointed time and inseminated their heifers. And guess what... their heifers calved without assistance, and their calves usually outgrew the calves out of their mature cows!

The bull I had been set up with was fantastic. All of the heifers that conceive to AI calved well before their due date with no calving troubles on any of the enrolled properties. Most of the producers fell in love with their calves, enrolled in a program for the upcoming joining, and talked to their friends. Essentially in this way, my AI book has grown to over 5,000 head per year and continues to grow.

What Does AI Cost?
An average bull may cost $6,000, be used for 3 years on average, and salvaged for $1,500 when sold. Whilst he is residing on the property, he will consume as much as a cow and a half, essentially displacing 1.5 cows and hence 1.35 calves, assuming a 90% conception rate overall. Calves average around $900 dollars. So a bull's rough annual costs are ($6000-$1500)/3 + 1.35 x $900 = $2715. If we divide a bulls annual running cost of $2715 by a booking of 40 heifers with a conception rate of 84% over six weeks ($2715/(40*0.84)) we come up with an annual cost of $81 per calf born over a roughly six week calving.

Roughly speaking, two rounds of AI using a new intravaginal progesterone device, reused for the second round costs about $30 in drug costs, $30 in semen costs, and $15 in professional fees, not including travel. This amounts to $75 with a presumed conception rate of 75%. This amounts to approximately $100 per calf born over roughly a three week calving.

$81 vs $100

However, the AI calves will on average be 10.5 days older due to synchrony, which works out to an additional 10 kgs of calf @ $3 per kilo...

$81 vs $70 ($100 - $30)

Further, the naturally covered heifers would likely have been covered by a low birthweight, low weaning weight, low accuracy bull... So let's conservatively assume 15 kgs @ $3 per kilo of additional weaned calf difference due to genetics...

$81 vs $25 ($70 - $45)

We aren't just saving money, we are making money, and we haven't even started talking about genetic gain! Worried about time..? shorter, tighter, calving with proven calving ease bulls... the time spent synchronising is far better than the time that could have been spent checking heifers and pulling calves!

What Else is in it for Producers?
Producers also benefit from capitalising on their best genetics, speeding their genetic turnaround from a three year interval when the calves from heifers weren't retained to a two year turn around, a huge 50% improvement in genetic gain! Calving becomes much more predictable, both by using proven bulls with predictable calving ease but also by condensing the joining into two rounds of Fixed Time AI separated by 23 days. The heifer progeny will hit the ground early and power away, often outweighing the progeny from the mature cows at weaning. Fixed Time Artificial Insemination isn't just affordable, its profitable!

Conclusion
Working closely with many of my producers to help them to improve their herd structure has ultimately led to the implementation of

Fixed Time AI programs over their heifers. I try to get them to keep more heifers and mate them over a short window, allowing the mating process to pick the winners. After all, reproduction is the key driver behind profitability. Ultimately, my goal is to help them to set their heifer up to succeed. By calving them down in a tight pattern with little or no need for assistance, setting them up for the next joining, we aren't just picking the best replacements, we are building better cows!

Practical Use of Embryo Transfer Technology in Beef Herds: Donor and Recipient Selection and Management

Gabriel A. Bó

Introduction

The objective of embryo transfer programs is to obtain the maximum number of transferable embryos with a high probability of producing pregnancies (Mapletoft and Bó, 2016). Superovulation has evolved greatly over the last years and is the best way of producing embryos in vivo. The development of purified pituitary extracts and progesterone-releasing (P4) devices have provided for the development of many of the protocols that we use today. Furthermore, the knowledge of follicular wave dynamics and real-time ultrasonography have provided practical approaches for the synchronisation of donors and recipients with protocols than can be simple to follow and with acceptable pregnancy rates, even without the necessity for oestrus detection. The objective of this article is to briefly review how we can select the best donors for superovulation and the most practical treatments to synchronise recipients and obtain the best results through embryo transfer.

Embryo Donor Selection

For many years cattle owners have used their own selection criteria for donor animals; however, in most cases it is economically driven. Nowadays, embryo transfer has changed from being applied only to only very valuable cattle for a more widespread use, even in commercial herds. This was mainly due to the simplification of the superovulation and recipient synchronisation treatments, as well as the embryo recovery, freezing and transfer techniques. Under these circumstances, the top 10% of a purebred herd could be used as donors while the lower 90% of the herd could be used as recipients at the beginning of the breeding season and those not conceiving bred by AI or by clean-up bulls, maintaining a yearly calving interval.

Although scarcity and promotion have tended to influence value, true genetic value, the ability to transmit desirable traits must be the most important long-range consideration. Selection should be based on three criteria: genetic superiority, reproductive ability and market value of the progeny. When selecting genetically superior beef donors, objective traits such as calving ease, milk production, weaning and yearling weights and carcass value should be considered. As beef bulls can now be evaluated for genetic merit quite accurately, the selection of the service sire is extremely important. Furthermore, genomic techniques are now being used to select embryo donors, and genomic analysis has become essential for the selection of bull dams used in embryo transfer (Ponsart et al., 2014; Seidel 2010). Although the technology still limited to some of the most popular breeds, the technology is expanding rapidly and it will become available for all breeds and even in cross-bred or synthetic breeds. It is predicted that within the next 10 years all embryo donors will be selected primarily on genomics.

As optimal results will also reduce costs, donor selection may involve a previous history of success in embryo transfer. In addition, daughters from cows that have been used successfully in embryo transfer are also likely to be successful. It has been suggested that the potential donor animal be at its prime reproductive age, have a history of a high level of fertility and demonstrated superiority in traits of economic importance. Strict selection criteria also include antral follicle counts. The numbers of antral follicles in the ovary as determined by ultrasonography has been shown to vary, and superstimulatory response has been shown to be correlated with the numbers of small antral follicles at the time of initiating FSH treatments (Ireland et al., 2007; Singh et al., 2004). Circulating antimullerian hormone (AMH) concentrations have been found to be an informative serum marker for ovarian follicle reserve, and information is accumulating that circulating AMH concentrations may be a reliable marker for predicting antral follicle numbers in cattle (Batista et al., 2014; Ireland et al., 2011; Monniaux et al., 2013). There was high repeatability across different phases of the oestrous cycle, days in milk, levels of milk production, and parities making AMH determinations particularly useful to select potential donors or to predict superovulatory response in selected donors (Souza et al., 2012).

Recipient Synchronisation

High pregnancy rates are partially dependent upon the onset of oestrus in recipients being within 24 h of synchrony with that of the embryo donor. The success of oestrus synchronisation programs is dependent on an understanding of oestrous cycle physiology, pharmacological agents and their effects on the oestrous cycle, and herd management factors that reduce anoestrus and increase conception rates.

Although, Prostaglandin $F_{2\alpha}$ (PGF) is still commonly used to synchronise recipients, it is not effective in inducing luteolysis in the first 5 days of the oestrus cycle and when luteolysis is effectively induced; the ensuing oestrus is not highly synchronised and distributed over a 6-day period (Kastelic et al., 1990). This is due to the status of the dominant follicle at the time of treatment. Therefore the success of these programs depends on the accuracy of oestrus detection and the availability of time of the practitioner to transfer the embryos 6 to 8 days after oestrus.

Fixed-Time Embryo Transfer (FTET)

The need for oestrus detection can be eliminated by utilising protocols that have been developed for fixed-time AI in cattle (Bó et al., 2012). Basically, the two approaches that have been used for embryo transfer are the so-called Ovsynch protocols or those based on oestradiol and P4-devices to synchronise follicle wave emergence and ovulation (Bó et al., 2012).

GnRH

GnRH-based protocols have been used to synchronise ovulation in recipients that received in vivo-derived (Baruselli et al., 2010; Hinshaw et al., 1999) or in vitro-produced (Ambrose et al., 1999) embryos. In these studies, more recipients received embryos than when oestrus detection was used because GnRH-based protocols do not depend on oestrus detection; thus, pregnancy rates are higher than in controls. However, the addition of a P4-device to a 7-day GnRH-based protocol is usually used for FTET; Hinshaw (1999) treated 1637 recipients with GnRH plus a P4-device and transferred in vivo-derived embryos, without oestrus detection, with an overall pregnancy rate of 59.9%.

Recent studies have shown that reducing the period of P4-device insertion and increasing the time from P4-device removal to GnRH improves pregnancy per AI in GnRH-based protocols (Bridges et al., 2008). This protocol is called 5-day Co-Synch and we have evidence indicating that this protocol results in a comparable proportion of recipients receiving an embryo and becoming pregnant per embryo transfer as with other FTET protocols (Bó et al., 2012) or oestrus detection (Sala et al., 2016). The two recommended protocols for FTET in bovine recipients using GnRH are shown in Figure 1.

Oestradiol and P4-devices

Oestradiol treatment protocols are the most commonly used treatment to synchronise follicle wave emergence and ovulation in beef and dairy recipients in South America (Baruselli et al., 2010; 2011). The P4-device is usually removed on Day 8 and ovulation is induced by the administration of 1 mg of oestradiol benzoate (ODB) 24 h after P4-device removal, or administration of GnRH 48 to 54 h after device removal (reviewed in Bó et al., 2012; Baruselli et al., 2010; 2011). As oestrus detection is usually not preformed, Day 10 is considered to be the day of oestrus. When oestrus detection is performed, all the recipients not in oestrus by 48 h after P4-device removal receive GnRH. All recipients with a functional CL on Day 17 receive an embryo; conception rates were comparable to embryo transfer 7 days after observed oestrus.

A common strategy to increase pregnancy rates in pasture-managed beef recipients in South America is the addition of 400 IU of eCG on either Day 5 or Day 8 of the oestradiol and P4 treatment protocol. Overall, 75 to 85% of the recipients treated with eCG receive an embryo (compared to 50% or less with simple PGF synchronisation), progesterone

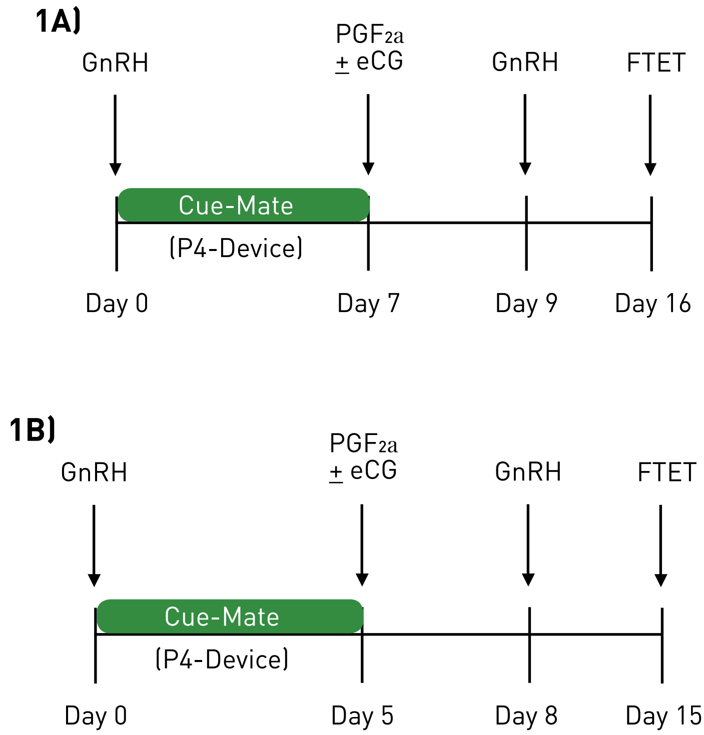

Figure 1. Two protocols for FTET in bovine recipients using GnRH. (A) Traditional 7-day GnRH+P4 device protocol (GnRH plus P4 device on Day 0 and GnRH on Day 9). (B) Modified 5-day GnRH+P4 device protocol (P4 device on Day 0 and GnRH on Day 8). Recipients with a CL detected by palpation or ultrasonography receive an embryo at a fixed-time (FTET) 7 days after GnRH. An injection of 400 IU of eCG may also be given to *Bos indicus* recipients or suckled *Bos taurus* recipients at the time of P4 device removal.

concentrations at the time of embryo transfer are high, and conception rates following transfer exceed 50% (reviewed in Baruselli et al., 2010; 2011; Bó et al., 2012).

The efficacy of the oestradiol benzoate, P4-device and eCG treatment protocol for FTET has been confirmed in several different parts of the world in more than 15,000 recipients (Argentina - Bó et al., 2005; Brazil - Nasser et al., 2011; China - Remillard et al., 2006; Mexico - Looney et al., 2010). In each of these studies, treatment with eCG increased the number of recipients receiving an embryo resulting in higher pregnancy rates.

A new protocol that has been recently investigated consist on the application of 2 mg ODB at P4-device insertion (Day 0), $PGF_{2\alpha}$ and 400 IU eCG is given at device removal on Day 6. No oestradiol is used to induce ovulation and recipients receive GnRH 72 h after device removal and an embryo 7 days later (Day 16). This protocol has been successfully evaluated in a series of experiments involving 3,893 recipients with pregnancy rates between 49% and 58% with *in vitro* produced embryos (Menchaca et al., 2016). The recommended protocols for FTET in recipients using oestradiol and P4-devices are shown in Figure 2.

Summary and Conclusions

Commercial embryo transfer in cattle has become a well-established industry. Although a smaller number of offspring are produced on an annual basis compared to AI, its impact is large because of the quality of animals being produced. Embryo transfer is now being used for genetic improvement,

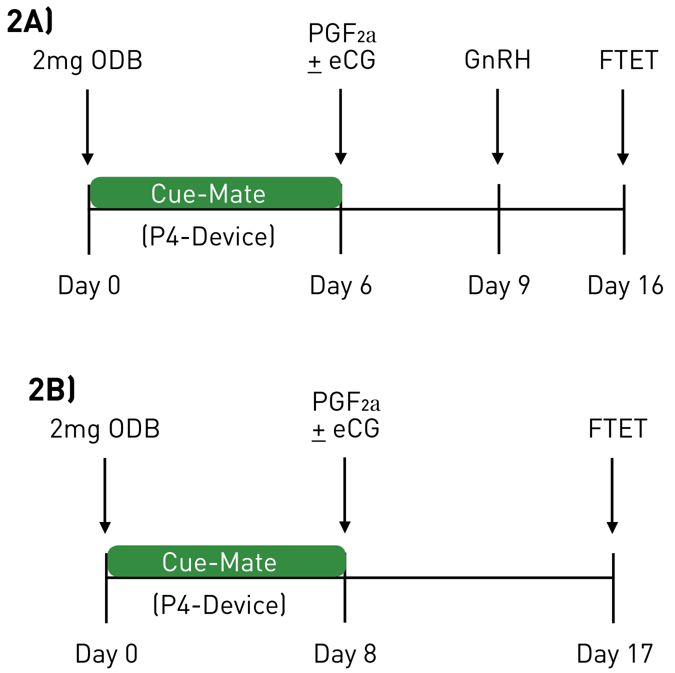

Figure 2. Oestradiol-based synchronisation protocols for FTET in bovine recipients. A) Conventional protocol: On Day 0, recipients receive a P4 device and oestradiol benzoate (ODB). On Day 8, P4 devices are removed and PGF is administered. Ovulation is induced with a second injection of ODB on Day 9 and fixed-time embryo transfer (FTET) is performed on Day 17. An injection of 400 IU of eCG may also be given to *Bos indicus* recipients or suckled *Bos taurus* recipients at the time of P4 device removal. If some oestrus detection is implemented with tail patches or tail paint, recipients not showing oestrus by 48 h after P4 device removal receive GnRH at that time. B) J-Synch protocol: P4-device insertion and ODB on Day 0; P4-device removal and PGF on Day 6; GnRH on Day 19; FTET on Day 16. If some oestrus detection is implemented with tail patches or tail paint, only recipients not showing oestrus by 72 h after P4-device removal receive GnRH at that time.

especially in the dairy industry, and most semen used today comes from bulls that have been produced by embryo transfer. An even greater benefit of bovine embryo transfer may be that *in vivo*-derived embryos can be made specified pathogen-free by washing procedures, making them ideal for control programs or in the international movement of animal genetics. Techniques have improved over the past 40 years so that frozen-thawed embryos can be transferred to suitable recipients as easily and simply as AI. A combination of embryo transfer with proven cows inseminated with semen from disease proven bulls, followed by industry-wide AI is a common commercial application of bovine embryo transfer.

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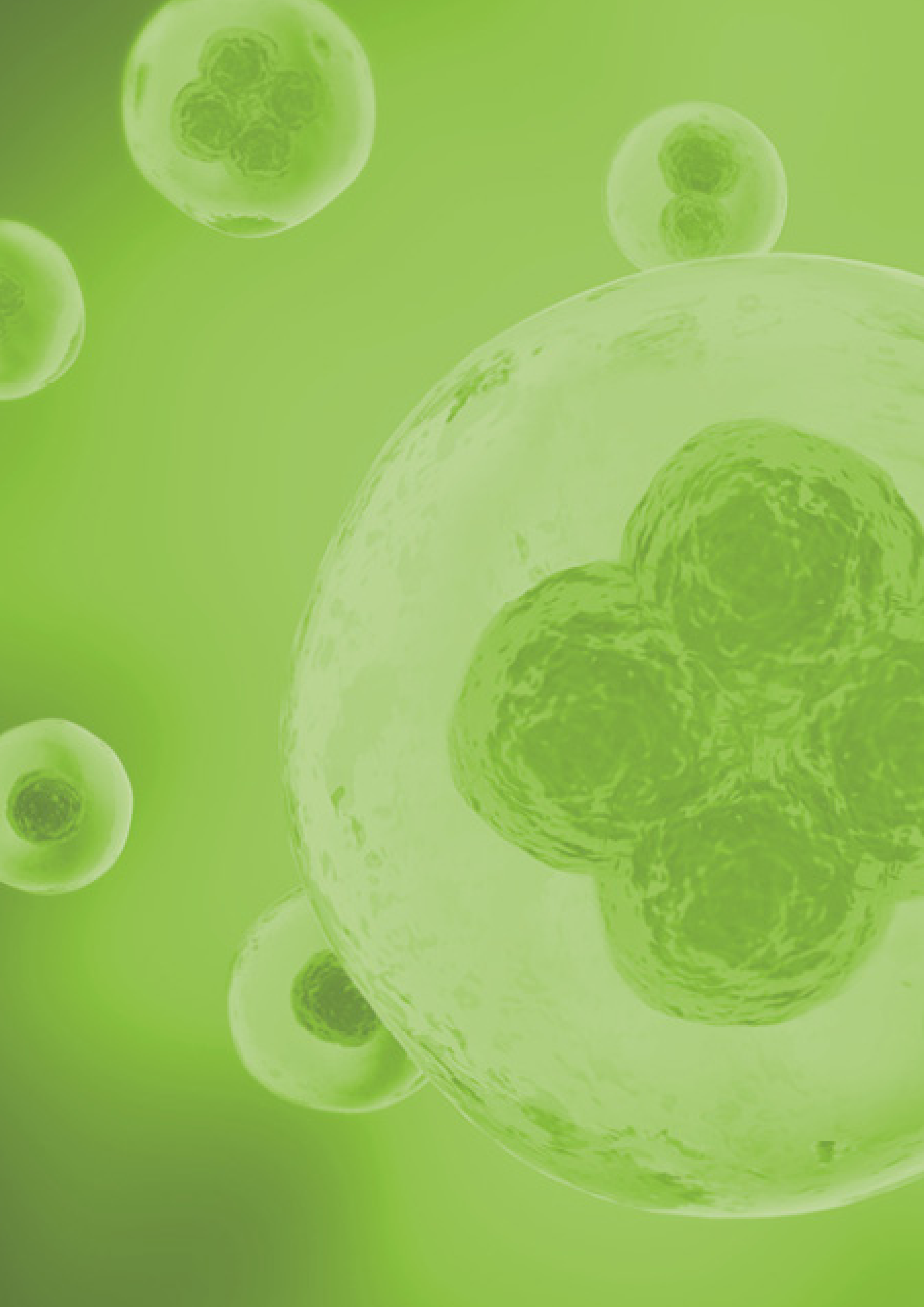

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