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State of the art in Bovine Reproduction Control and Artificial Insemination

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Abstract

The development of fixed-time artificial insemination (FTAI) protocols beginning in the late 20th century has led to an exponential increase in the number of animals inseminated worldwide, especially in South America. One of the main reasons for the growth of FTAI has been the possibility of inseminating postpartum anestrus cows and prepubertal heifers at the beginning of the breeding season. Most FTAI treatments in South America are based on the use of intravaginal devices impregnated with progesterone (P4) and estradiol (E_2) to synchronize follicular development and ovulation. However, these protocols are currently at risk due to restrictions on estradiol use by the European Union. The most apparent alternative is the application of GnRH-based protocols, which have a slightly different physiological basis than estradiol administration, but which have been used in other countries for some time. However, these protocols need to be adapted to the physiological conditions of our cows and heifers, both for Bos taurus, adapted to temperate zones, and for Bos indicus adapted to tropical environments. Therefore, the challenge is already set, and we must learn to use alternative protocols to continue increasing the use of this technology in our beef and dairy herds.

Keywords: Timed AI, Progesterone, estradiol, GnRH, eCG.

Introduction

Increased knowledge of ovarian physiology in cattle over the last 50 years has enabled the development of treatments for manipulating ovarian function. Protocols designed to monitor luteal and follicular function have led to the development of fixed-time artificial insemination (FTAI) and fixed-time embryo transfer (FTET) protocols. The objective of this review is to present some alternative protocols based on progesterone-releasing devices (P4) and estradiol or GnRH studied by our working group and to discuss the challenges of using these alternative protocols in the field.

The Use of Progesterone (P4) for the induction of estrus and ovulation

The administration of exogenous P4 alters ovarian function in cattle by suppressing estrus and preventing ovulation, primarily by suppressing the release of the preovulatory LH surge. Since P4 does not suppress FSH secretion, follicular waves continue to emerge in the presence of a functioning CL (Adams, 1999). When P4 is administered at intervals exceeding the lifespan of a CL, it results in synchronous estrus upon withdrawal, but fertility is low because the commercially available intravaginal devices that release P4 produce subluteal levels of P4 that are less suppressive of LH pulsatility than the endogenous P4 produced by the CL. The resulting high frequency of LH pulses leads to a "persistent dominant" follicle (Savio et al., 1993) with an aged or pre-activated oocyte that may be infertile or, if fertilized, results in early embryonic death (Revah and Butler, 1996).

Estradiol Esters

Estradiol valerate was the first estradiol salt originally used at the beginning of a 9-day norgestomet implant protocol (called Syncro-Mate-B), and its objective was to induce uterine-induced

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luteolysis (Wiltbank et al., 1965). We subsequently demonstrated that estradiol valerate also suppresses antral follicles (Bó et al., 1991). The mechanism of estrogen- and P4-induced follicular atresia involves suppression of FSH and LH (Bó et al., 1994; 1995). Once the exogenously administered estradiol is metabolized, FSH levels rise and a new follicular wave develops (Bó et al., 1994). Administration of 5 mg of estradiol-17β (E-17β; Bó et al., 1994) or 2 mg of estradiol benzoate (EB; Martínez et al., 2005) or estradiol valerate (EV; Colazo et al., 2005) to cattle treated with P4 resulted in the emergence of a new follicular wave within 3 to 5 days.

With this basic knowledge, the estradiol and P4-based TAI protocols that are used nowadays were developed. In this protocols, 2 mg of EB is administered at the time of insertion of a P4 device, which is removed 7, 8 or 9 days later at the time of prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) administration (Mapletoft et al., 2003; Bó et al., 2013). Initially, the recommendation was to administer a dose of 1 mg of EB 24 hours later to induce a synchronous preovulatory LH surge (Martínez et al., 2007) and ovulation. This allowed for FTAI with acceptable pregnancy rates. As an alternative, the administration of 0.5 to 1.0 mg of estradiol cypionate (ECP; Colazo et al., 2003) was later incorporated. ECP is an estradiol salt with a longer half-life than EB and reaches lower plasma concentrations of estradiol-17β than EB. Therefore, it can be adapted to a regimen of ECP administration as an ovulation inducer at the time of device removal with P4 (Colazo et al., 2003 and 2004; Sá Filho et al., 2011; Sales et al., 2012; Uslenghi et al., 2014; Madureira et al., 2020; Cedeño et al., 2021a). Therefore, the use of ECP at the time of P4 device removal with P4 is now the most widely used treatment to reduce the number of animal handlings. The current recommendation is to use 1 mg of ECP in suckled beef cows and lactating dairy cows and 0.5 mg in heifers.

Another important addition to the FTAI protocols has been the use of equine chorionic gonadotropin (eCG), which is injected at the time of P4 device removal. In its natural form, this hormone is a high-molecular-weight glycoprotein produced by the endometrial cups of the mare's uterus between 35 and 100 days of gestation. In the mare, eCG has LH activity, but in the cow, eCG can have FSH or LH activity, depending on the receptor populations in the ovarian follicles at that time. Although eCG was originally used to induce superovulation in cattle, its use in cattle is now more oriented toward stimulating the growth of the dominant follicle in AI programs and embryo recipients (Bó et al., 2002; 2016; Baruselli et al., 2012; 2017). When administered to cows with a growing dominant follicle, eCG stimulates follicular growth because it can bind to both LH and FSH receptors. Greater growth of the ovulatory follicle and higher ovulation rates result in a larger CL and higher pregnancy rates (*Bos taurus*, Núñez-Olivera et al., 2014; *Bos indicus*, Baruselli et al., 2004).

Until recently, 100% of the eCG used in cattle was produced through the bleeding of pregnant mares; however, we now can produce this hormone in the laboratory using cell cultures grown under controlled conditions using fetal bovine serum-free culture media. For this reason, these hormones are generically called "recombinant." Today we have at least one recombinant eCG on the market, and recent experiments have shown that the addition of recombinant eCG increases pregnancy rates in suckled cows (Villarraza et al., 2022; Cattaneo et al., 2024). With some modifications in the dosage used, no significant differences were found with serum eCG derived from mare bleeding. Other recombinant eCGs are about to appear on the market with similar results (de Abreu et al., 2023), and it is expected that in the future, recombinant and/or synthetic hormones will replace those obtained from animals due to political pressure from environmental groups.

The J-Synch Protocol

Approximately 13 years ago, we developed a treatment using an EB-based protocol and a P4 device in which proestrus was prolonged by not giving ECP at device removal and only administering GnRH to advance the time of ovulation in those heifers not showing estrus by 66-72 hours after device removal. This protocol was named J-Synch (de la Mata and Bó, 2012; de la Mata et al., 2018). It was found that pregnancy rates at FTAI were higher than those obtained with the conventional protocol (with ECP or EB as ovulation inducers) in Holstein heifers (Ré et al., 2021), beef heifers, and embryo recipients (Bó et al., 2016; 2018; 2022; Cedeño et al., 2019, 2021b and 2022; de la Mata et al., 2018; Frutos et al., 2018; Menchaca et al., 2016; Motta et al., 2016; Pincinato et al., 2018; Pereira et al., 2022). This protocol promotes modifications during the proestrus period that are positively associated with an improved uterine environment (de la Mata et al., 2018; Núñez-Olivera et al., 2022). The initial recommendation was to use 300 IU of eCG at the time of P4 device removal in beef heifers (Núñez-Olivera et al., 2020), but the current recommendation is to use 200 IU of serum eCG (Cuadro et al., 2024) or 49 to 70 IU of recombinant eCG to avoid a high twinning rate. The reason for the higher twinning rate associated with high dosages of eCG is due to the length of the P4-device insertion period (6 days), which is only two days after wave emergence, and probably the subordinate follicles are not completely suppressed by the growing dominant follicle in some heifers.

Therefore, we have recently conducted two experiments in dairy heifers to evaluate whether it was possible to achieve acceptable pregnancy rates by prolonging the P4-device insertion period to 7 days and without using eCG. In the first experiment, 37 Holstein heifers that were 18.8 ± 0.2 months of age and all cycling (with a CL detected by ultrasonography) were randomly assigned to two treatments: 6-day J-Synch or 7-day J-Synch. Heifers in the 6-day J-Synch group received 2 mg of EB (Estradiol, Over, Argentina) intramuscularly (i.m.) and a device containing 0.7 g of P4 (Sincrover, Over) on Day 0. On Day 6, the devices were removed, and 150 µg of D (+) cloprostenol (Prostal, Over) were administered. Those in the 7-day J-Synch group were treated the same as above, except that the device remained in place for 7 days. Heifers in both groups were painted for estrus detection at the time of P4 device removal and underwent ultrasonography on Day 0 and every 12 h thereafter from device removal until ovulation. Ovulation rates did not differ (6-day J-Synch 77%, 14/18, and 7-day J-Synch 100%, 19/19), and heifers had a device removal-to-ovulation interval of 102.0 ± 6.3 h and 92.2 ± 5.4 h for the 6- and 7-day J-Synch, respectively (P = 0.2). However, ovulatory follicle diameter was larger (P = 0.02) in the 7-day J-Synch group (13.6 ± 0.5 mm) than in the 6-day J-Synch group (15.2 ± 0.4 mm). In the second experiment, 638 heifers of the same age as those in Experiment 1 were used and assigned to the same treatments. The difference was that all heifers that were >30% unpainted at 72 h after removal of the devices were AI with sexed semen at that time, and those that were not unpainted (not in heat) received 10 µg of buserelin (Gestar, Over) and were AI with sexed semen 12 h later. In this case, the overall estrus expression was 83.8% (535/638) and did not differ (P = 0.3) between groups (J-Synch 6 days: 82.4%, 263/319; J-Synch 7 days: 85.3%, 272/319). However, pregnancy rates were higher (P=0.02) in heifers in the 7-day J-Synch group (44.5%, 142/319) than in those in the 6-day J-Synch group (35.7%, 114/319). In conclusion, we can indicate that removal of the device one day later results in a larger size of the dominant ovulatory follicle and a higher pregnancy rate with sexed semen. Therefore, of the two protocols evaluated, the 7-day J-Synch is more effective for inseminating Holstein heifers with sexed semen without the use of eCG.

Gonadotropin releasing hormone (GnRH)

GnRH treatment in dairy cows with a viable dominant follicle will induce an LH surge and ovulation (Macmillan and Thatcher, 1991). However, GnRH will only synchronize follicular development when ovulation of the dominant follicle occurs (Martinez et al., 1999). Pursley et al. (1995) developed the Ovsynch protocol, which today, with several modifications, is the most widely used protocol in dairy cattle worldwide (Cosentini et al., 2021).

In beef cattle, GnRH-based protocols are used in North America, Europe, New Zealand, and Uruguay, due to restrictions on the use of estradiol in animals whose meat will be consumed in those countries. The first modification to the dairy protocol to make it suitable for beef cows was to simplify it by placing the second GnRH at the time of the FTAI (a protocol called Co-Synch; Geary et al., 2001a). Furthermore, Co-Synch protocols include the insertion of a P4 device to overcome low ovulation rates after the first GnRH in heifers (Martinez et al., 2002) and in postpartum anestrus cows (Lamb et al., 2001; Gunn et al., 2016).

The addition of eCG to GnRH-based treatment protocols and P4 devices has also been reported to improve pregnancy rates at FTAI in postpartum anestrus cows (Pincinato et al., 2012; Huguenine et al., 2013). In the Northern Hemisphere, improved pregnancy rates have been reported in *Bos taurus* cows with a high rate of anestrus in Ireland (Randi et al., 2021) and in primiparous *Bos taurus* cows in Canada (Small et al., 2009). However, no improvements in pregnancy rates have been reported in *Bos taurus* cows with a low incidence of postpartum anestrus and good body condition that also received a very low dose of eCG (200 IU, Marquezini et al., 2013). The largest difference between the positive and negative studies is the dose of eCG used (400 IU in Randi et al., 2021, and Small et al., 2009, and 200 IU in Marquezini et al., 2013). GnRH protocols have also evolved, and new prolonged proestrus protocols have been developed with the goal of increasing the period of preovulatory estradiol exposure and improving uterine function and early embryo development (Bridges et al., 2008; 2012; 2014). Greater preovulatory estradiol exposure was also associated with lower embryonic loss between pregnancy recognition and placental membrane adhesion (Madsen et al., 2015). The protocol was called the 5-day Co-Synch+P4 protocol and resulted in a higher pregnancy rate than the 7-day Co-Synch+P4 protocol in beef cows (Bridges et al., 2008; Whittier et al., 2013). Because of the shorter interval between the first GnRH and the induction of luteolysis in the 5-day Co-Synch+P4 protocol, it is recommended to use two doses of PGF 6 to 24 h apart in cows (Souto et al., 2009). In an experiment with 2,465 postpartum beef cows, the pregnancy rate was higher (P<0.05) in cows that received two PGF 8 h apart (55%) than in those that received only one PGF (48%), and those that received two PGF 8 h apart had an intermediate pregnancy rate (51%; Bridges et al., 2012). Therefore, double PGF 8 to 24 h apart appears to be necessary to maximize fertility in cows with the 5-day protocol. However, if herd management conditions do not allow for the additional handling, a double dose of PGF 8 h apart would be an acceptable alternative.

This protocol has also been extensively evaluated in Uruguay (Garcia Pintos et al., 2022), where it was also decided to slightly alter the 5-day Co-Synch+P4 protocol by modifying the timing of device removal and the FTAI (a protocol called 5-day Split-Synch). This facilitates the administration of PGF, giving the first dose at the time of device removal and the eCG on Day 5 PM. The cows are then kept in the corral overnight and receive the second PGF before being released the following morning. Paint is also placed at the base of the tail to detect those already in heat at the time of the FTAI. Cows that had >30% of the paint rubbed off (i.e., in estrus) are FTAI starting at approximately 62 h, and those without the paint rubbed off receive the second GnRH and are FTAI in the afternoon (Figure 1). The same protocol was used in heifers, and it was reported that in this category, a dose of GnRH is required at the start of treatment, but only a dose of $PGF_{2\alpha}$ is required at the end of treatment to induce luteolysis. Most importantly, the results in heifers were similar to those obtained with the J-Synch protocol, and in cows, they were similar to those obtained with the conventional protocol with ECP as the ovulation inducer.

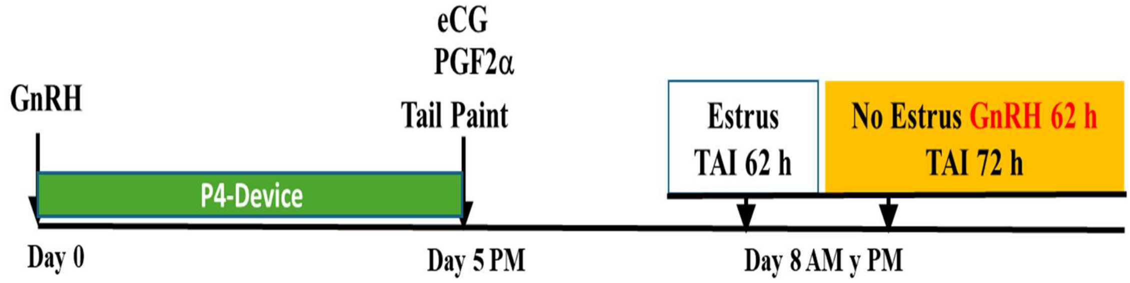


Figure 1. 5-day Split-Synch protocol. In cows, the second PGF injection could be omitted, and the recommended eCG dose is 200 IU (Adapted from Garcia Pintos et al., 2022).

The 5-day Co-Synch+P4 protocol has also been investigated in Bos indicus cows in Brazil, with a lower pregnancy rate in suckled Nelore cows than those treated with the conventional estradiol/P4-based protocol (Ferraz Jr et al., 2016). An important difference was that 400 IU of eCG was used in the estradiol/P4-based protocol, but not in the 5-day Co-Synch+P4 protocol. To confirm this notion, we found a higher pregnancy rate in postpartum anestrous cows receiving 400 IU of eCG upon device removal with P4 (5-day Co-Synch+P4: 46.3%, 120/259) than in cows treated with the 5-day Co-Synch+P4 protocol but without eCG (26.8%, 71/265; $P < 0.05$; Huguenine et al., 2013). At the same time, the pregnancy rates of the Co-Synch plus eCG protocol and the conventional one with ECP were similar (46.3% for Co-Synch+P4+eCG and 54.5% for the protocol with EB+P4+ECP+eCG, respectively). We also recently conducted an experiment to evaluate pregnancy rates at FTAI in *Bos indicus* crossbred cows synchronized with protocols similar to those evaluated in Uruguay (Split-Synch). However, in this case, cows that were >30% unpainted (in heat) were inseminated 72 h after device removal, and those that were not in heat received GnRH at 72 h and were inseminated 8–10 h later (Bó et al., 2023). Suckled Cows with a CL or at least one follicle ≥8 mm in diameter, body condition score between 2 and 4 (scale 1 to 5) were synchronized with one of three treatments: 7-day J-Synch, 6-day Split-Synch, and 5-day Split-Synch. In both Split-Synch treatments, two doses of $PGF_{2\alpha}$ were applied 12 h apart. The pregnancy rate was higher ($P=0.01$) in cows in the 7-day J-Synch group (55.0%, 228/415) than in the 6-day Split-Synch (45.0%, 167/370) and 5-day Split-Synch (38.5%, 145/376) groups. In conclusion, the higher pregnancy rate obtained with the estradiol-based protocol suggests that further improvements in GnRH-based protocols are needed to improve pregnancy rates in *Bos indicus* beef cows. The difference is probably due to the lower effectiveness of GnRH than EB in synchronizing the growth of a new follicular wave, and it may be necessary to double the GnRH dose, since the magnitude of the LH surge produced by GnRH administration is lower in *Bos indicus* than in *Bos taurus,* especially in cows with a CL (Batista et al., 2017).

Web-Synch Protocol

One of the main limitations for the application of GnRH protocols in beef cows and heifers is the low response to the first dose of GnRH (Geary et al., 2001b, Martinez et al., 2000). The other limitation under the anestrus conditions prevalent in beef herds in South America is the need to use eCG in anestrus cows to obtain pregnancy rates close to 50% (Bó et al., 2016). Recently, Bonacker et al. (2020) developed a new synchronization protocol called 7 & 7 Synch, lasting 17 days and consisting of 4 handlings, using previous knowledge generated by Small et al. (2009). This protocol consists of applying $PGF_{2\alpha}$ and a device with P4 on Day -17 as pre-synchronization to develop a persistent follicle; on Day -10, GnRH is administered to ovulate the present persistent follicle; on Day -3, a luteolytic dose of $PGF_{2\alpha}$ and device removal, and finally FTAI together with a dose of GnRH on Day 0 (60 to 66 hours after device removal). The 7 & 7 Synch protocol demonstrated an improvement in the ovulatory response to the first administration of GnRH (Bonacker et al., 2020) and greater pregnancy rates compared to a 7-day Co-Synch + P4 in beef cows (Andersen et al., 2021). This protocol also resulted in greater pregnancy rates than the 7-day Co-Synch + P4 protocol and the conventional estradiol protocol in Angus cows in Argentina (Ferré et al., 2023).

Based on this knowledge and given the potential restrictions on estradiol use, an alternative GnRH-based treatment was designed, which we called "Web-Synch" (Figure 2). On Day 0, cows receive a pre-synchronization treatment with $PGF_{2\alpha}$ and the insertion of a P4 device to generate a persistent follicle. On Day 5, GnRH is injected to induce ovulation of the persistent follicle and to promote the emergence of a new follicular wave (36 hours later). Subsequently, on Day 11, the device is removed along with a dose of $PGF_{2\alpha}$ and eCG to induce follicular growth and promote a prolonged proestrus, as in the J-Synch and the 5-day Co-Synch treatments. Lactating dairy cows also receive a second $PGF_{2\alpha}$ on Day 12. Finally, the FTAI is performed 72 hours after device removal (Day 14), with GnRH administered only to animals not in estrus at that time.

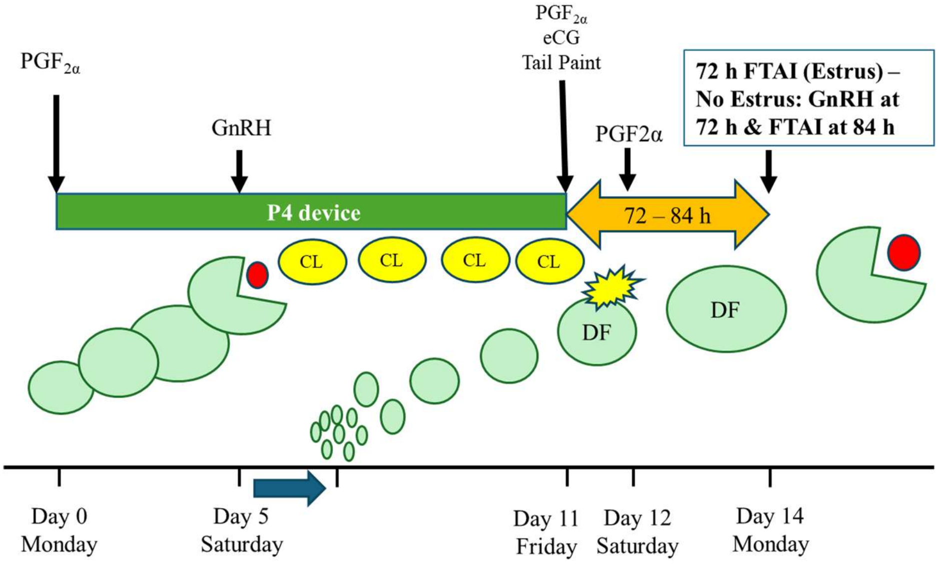


Figure 2. Diagram of the Web-Synch protocol. Based on the results obtained so far, in beef cows it is still unclear whether a double dose of GnRH should be used on Day 0 to induce ovulation and whether one or two doses of PGF2α are necessary, while in dairy cows a high ovulation rate (66.9%) has been observed using a single dose (10 µg of buserelin) and from the results of previous work we know that two doses of PGF2α are necessary to induce complete luteolysis in high-producing cows.

A series of experiments were conducted to evaluate the Web-Synch protocol in beef cows (de la Mata et al., 2022). In Experiment 1, cows (n=52) were randomly divided to receive three treatments. Cows in the Web-Synch treatment (n=20) received an intravaginal device containing P4 (CIDR-B 1.9 g P4, Zoetis) along with 150 µg D-cloprostenol ($PGF_{2\alpha}$, Veteglan, Calier) i.m. on Day 0 and 20 µg buserelin acetate (GnRH, Buserelin, CEVA) on Day 5. On Day 11, the device was removed from all cows, along with the i.m. administration of $PGF_{2\alpha}$ and 105 IU of reCG (Foli-Rec, CEVA). Cows in the J-Synch group (n=17) received CIDR-B along with 2 mg EB (estradiol benzoate, Calier) i.m. on Day 0. On Day 6, the device was removed from all cows, along with the i.m. administration of $PGF_{2\alpha}$ and 105 IU of reCG (Foli-Rec, CEVA). Cows in the 6-day Co-Synch + P4 group (n=20) received CIDR-B on Day 0 together with a 10 µg i.m. dose of GnRH. On Day 6, the device was removed from all cows, along with the i.m. administration of $PGF_{2\alpha}$ and 105 IU of reCG (Foli-Rec, CEVA). In addition, all cows in the three treatment groups received heat detection patches (Fasco AP, Argentina), and those showing estrus by 72 h after P4 device removal were FTAI at that time. Those that did not show estrus received GnRH and were also inseminated at 72 h after P4 device removal. Cows were monitored by transrectal ultrasonography to determine follicular diameter, ovulatory rate, and post-ovulatory CL size. In Experiment 2, cows (n=19) were randomly divided into four treatments in a 2 x 2 factorial experimental design and synchronized with the Web-Synch protocol or the J-Synch protocol with either and intravaginal device with 0.7 g of P4 (Sincrover, Over; n=5) or with 1.0 g of P4 (Sincrover, Over; n=5). The cows were inseminated and monitored by ultrasonography, as in Experiment 1, to determine follicular growth, time of ovulation, and ovulatory rate. In Experiment 3, suckled cows (n=213) were divided as in Experiment 2 to evaluate pregnancy rates to FTAI. In Experiment 1, ovulation rates did not vary among treatments, being 100, 83.3, and 80.0% for the Web-Synch, 6-day Co-Synch, and J-Synch groups, respectively (P>0.1). Follicular diameter at FTAI was greater in the Web-Synch and 6-day Co-Synch treatments (11.8 ± 0.4 and 11.7 ± 0.5 mm, respectively) than in the J-Synch treatment (9.8 ± 0.6 mm; P<0.05). In Experiment 2, no differences (P>0.1) were observed in ovulatory rate and timing between treatments, being 80.0% and 94.5 ± 3.5 hours for the Web-Synch treatment and 100% and 96.7 ± 5.1 hours for the J-Synch treatment (P>0.1). In Experiment 3, both estrus rate and pregnancy rate did not differ between treatments, being 66.0 and 58.7% for Web-Synch, and 58.0 and 54.1% for J-Synch (P>0.1), respectively.

More recently, other experiments were conducted. In the first experiment, 170 suckled Angus cows with 40 to 65 days postpartum were used, with 51.1% of the herd presenting a CL by ultrasonography (de la Mata et al., 2023a). The cows were randomly allocated into 4 treatment groups, in 2 x 2 factorial arrangement to receive the Web-Synch protocol or the conventional estradiol/P4-based protocol, using an intravaginal devices containing 0.6 or 1.2 g of P4 (Pluselar; Calier, Argentina). Cows in the Web-Synch group were treated as those in previous experiments, whereas those in the conventional group received 2 mg of estradiol benzoate (EB, Calier, Argentina) with a Pluselar 0.6 or a Pluselar 1.2 device on Day 0. On Day 7, the devices were removed along with a dose of $PGF_{2\alpha}$, 105 IU reCG, and 1 mg of estradiol cypionate (Calier, Argentina). As in the previous experiments, all cows received an estrus detection patch. Cows that showed estrus 72 h (Web-Synch) or 48 h (Conventional) after P4 device removal (patch rubbed off >50%) were FTAI at that time, whereas those that did not (patch not rubbed off) received 10 µg of GnRH and were FTAI at that time. The pregnancy rate did not vary between treatments, being 44.3% (39/88) in those receiving the Web-Synch and 45.1% (37/82; P = 0.3) in those receiving the conventional treatment. In the second experiment, 304 suckled Angus cows, with 40 to 65 days postpartum, were used. In this case only 9.8% of the cows had a CL on Day 0 (de la Mata et al., 2023). The cows were randomly divided into a 2 x 2 factorial experimental design as in the previous experiment, and the pregnancy rate was higher in cows that received the conventional treatment (66.3%; 102/154) compared to those that received the Web-Synch treatment (49.4%; 79/160; P = 0.01). Initially, the conclusion of this last experiment was that when herds with a high degree of anestrus are synchronized, the pregnancy rate is higher in cows treated with conventional estradiol treatment. However, in another experiment recently conducted in Paraguay, no significant differences were found between treatments, and the cyclicity rate was 5.3%. Finally, in a recent experiment with Brangus cows and a high cyclicity rate, no significant differences were found between the Web-Synch group and the conventional group. The results of all these experiments are summarized in Table 1. Adding all the cows evaluated, the pregnancy rate did not differ between among treatments.

Finally, experiments were recently conducted in lactating Holstein cows to evaluate ovulatory follicle size, ovulation timing, and pregnancy rates at FTAI in lactating Holstein cows synchronized with either the Web-Synch protocol or the conventional estradiol protocol. In the preliminary experiment (Macagno et al., 2022), lactating Holstein cows (n=179), 41.3±0.9 kg of milk per day, 139.0±8.3 days in milk, 1.9±0.1 lactations, body condition score of 3.0±0.1 (scale 1 to 5), and managed in a "Dry Lot" system were randomly assigned to one of two treatment groups. On Day 0, cows in Group 1 (J-Synch) received 2 mg of EB (Estradiol, Over, Argentina) and an intravaginal device with 1 g of P4 (Sincrover, Over). On Day 6, the P4 devices were removed, and cows received 150 µg D-cloprostenol ($PGF_{2\alpha}$, Prostal, Over) and 400 IU eCG (Novormon, Zoetis). On Day 7, a second dose of $PGF_{2\alpha}$ was administered. Cows in Group 2 (Web-Synch) were treated with $PGF_{2\alpha}$ and a P4 device on Day -5 and 20 µg of buserelin (GnRH, Gestar, Over) on Day 0; device removal, $PGF_{2\alpha}$ and eCG were performed on Day 6, and a second dose of $PGF_{2\alpha}$ was administered on Day 7. Cows in both groups were painted at the base of their tail for estrus detection and were FTAI 80 h after P4 device removal. Cows with tail paint removed <30% also received 10 µg of GnRH at that time. All cows were examined by ultrasonography on Day -5 to determine cyclicity, on Days 0 and 6 to determine GnRH ovulation in the Web-Synch group, and at 30 days post-FTAI for pregnancy diagnosis. Overall, estrus expression was 49.7% (89/179), and pregnancy rate did not differ (P>0.6) depending on whether cows were in (42.6%, 38/89) or not (36.6%, 33/90) in estrus at the time of FTAI. However, both estrus expression and pregnancy rate were higher (P<0.05) in cows in the Web-Synch group (59.3%, 54/91, and 49.4%, 45/91, respectively) than in those in the J-Synch group (39.7%, 35/88, and 29.5%, 26/88, respectively). The proportion of cows with CL on Day 5 did not differ between groups (J-Synch: 60.2%, 53/88 vs. Web-Synch: 63.7%, 58/91; P>0.6). However, the proportion of cows with CL in the Web-Synch group was 12.1% on Day 0 and 76.9% on Day 6, indicating that at least 64.8% of cows ovulated in response to the first administration of GnRH.

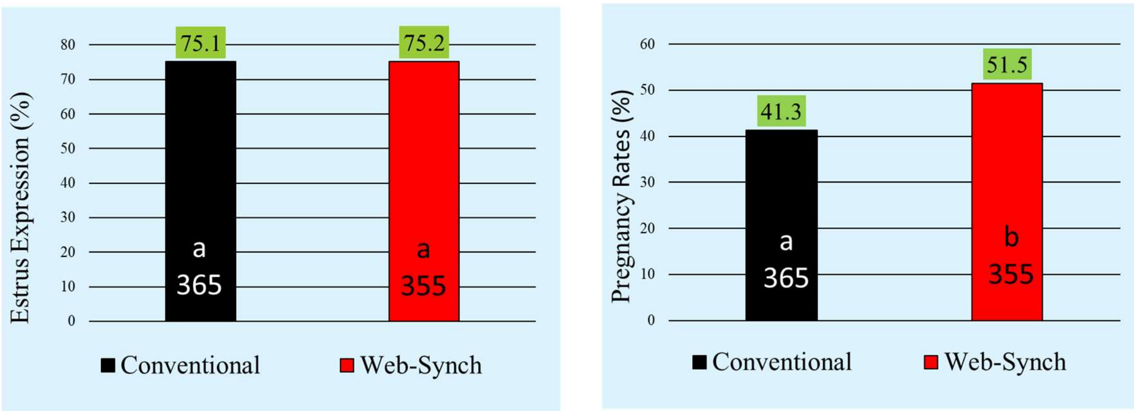
Table 1. Pregnancy rates to FTAI in beef cows synchronized with estradiol/P4 based protocols or the Web-Synch protocol.

Reference	**Cows with CL o Day 0**	**Control**	**Web-Synch**	**P Value**
de la Mata et al., 2022[1]	43,8%	11/15 73,3%	12/19 63,1%	NS
de la Mata et al., 2022[1]	100%	8/10 (80%)	5/10 50%	NS
de la Mata et al., 2022[1]	62,7%	59/109 (54.1%)	61/104 (58.7%)	NS
de la Mata et al., 2023[2]	51,1%	37/82 (45,1%)	39/88 (44,3%)	NS
Frutos et al., 2024[2]	5,3%	45/104 (43,3%)	45/103 (43,7%)	NS
Bonfil et al., 2024[2]	42,9%	54/107 (50,5%)	55/105 (52,4%)	NS
de la Mata et al., 2023[2]	9,8%	102/154 (66,3%)	79/160 (49,4%)	P<0,01
Total		316/581 (54,4%)	296/589 (50.3%)	NS

[1]Control Group: J-Synch
[2]Control Group: Conventional with ECP

Following this preliminary experiment, two further experiments were conducted with lactating Holstein cows (n=39 in Experiment 1 and 720 in Experiment 2). In this case, the cows used were 160.0±7.1 days in milk, producing 35.6±0.8 kg of milk per day, with a 2.8±0.3 lactation period, with a body condition score of 3.1±0.1, and were managed in a dry-lot system. Cows were randomly assigned to one of two treatment groups. On Day 0, cows in the control group received 2 mg of EB (Estradiol, Over, Argentina) and an intravaginal device containing 1 g of P4 (Sincrover, Over). On Day 6, cows received 150 µg D (+) cloprostenol ($PGF_{2\alpha}$, Prostal, Over). On Day 7, the P4 devices were removed, and cows received a second dose of $PGF_{2\alpha}$, 140 IU of reCG (FoliRec, CEVA), and 1 mg of ECP (Estrosinc, Over). Cows in the Web-Synch group were treated with $PGF_{2\alpha}$ and a P4 device on Day -5 and 10 µg of buserelin (GnRH, Gestar, Over) on Day 0. The removal of the P4 devices, $PGF_{2\alpha}$, and reCG was performed on Day 6, and a second dose of $PGF_{2\alpha}$ was administered on Day 7. Cows in both groups were painted at the base of their tail for estrus detection. In Experiment 1, cows were scanned twice daily from P4 device removal until ovulation. In Experiment 2, all cows that had >30% of their tail paint removed on Day 9 (48 h after P4 device removal in the control group and 72 h after P4 device removal in the Web-Synch group) were AI at that time, and cows that had not had tail paint removed in both groups received 10 µg of GnRH and were AI 12 h later. Cows in Experiment 2 were examined for pregnancy 30 d after AI. In Experiment 1, the mean (±SEM) interval from P4 device removal to ovulation was longer (P<0.05) in the Web-Synch group (101.6±2.9 h) than in the control group (78.3±3.1 h), but the diameter of the ovulatory follicle did not differ (P=0.3; 19.7±0.8 and 18.5±0.8 mm for the Web-Synch and Control groups, respectively). In Experiment 2, although no significant differences in estrus expression (P=0.3) were found between the Web-Synch (75.2%) and Conventional (75.1%) groups, the pregnancy rate was higher (P<0.01) in the Web-Synch group (51.5%, 183/355) than in the Conventional group (41.3%, 151/365), respectively (Macagno et al., 2023; Figure 3). Furthermore, 66.5% of cows (236/355) ovulated at the first GnRH in the Web-Synch protocol. In summary, the GnRH-based synchronization protocol (Web-Synch) resulted in a longer proestrus period and a higher pregnancy rate than the conventional estradiol-based protocol in lactating dairy cows.

Figure 3. Estrus expression and pregnancy rates in lactating Holstein cows synchronized with the Web-Synch or the conventional estradiol/P4 based protocol. Bars with different letters differ (P<0.01).



Final Comments

Undoubtedly, advances in our understanding of cow reproductive physiology will allow us to face the upcoming challenges in the implementation of reproductive technologies in beef and dairy cattle. Our obligation will always be to maximize productivity to produce low-cost food for a growing population. However, we must implement practices that are accepted by 21st-century consumers and, above all, demonstrate that we can feed the world with safe methods, animal welfare, and environmental protection.

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References

Adams GP. Comparative patterns of follicle development and selection in ruminants. *J Reprod Fertil Suppl.* 1999;54:17-32.

Andersen CM, Bonacker RC, Smith EG, Spinka CM, Poock SE, Thomas JM. Evaluation of the 7 & 7 Synch and 7-day CO-Synch + CIDR treatment regimens for control of the estrous cycle among beef cows prior to fixed-time artificial insemination with conventional or sex-sorted semen. *Anim Reprod Sci.* 2021;235:106892.

Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim Reprod Sci*. 2004;82-83:479-486.

Baruselli PS, Sá Filho MF, Ferreira RM, Sales JNS, Gimenes LU, Vieira LM, Mendanha MF, Bó

GA. Manipulation of follicle development to ensure optimal oocyte quality and conception rates in cattle. *Reprod Dom Anim.* 2012;47:134-141.
Baruselli PS, Ferreira RM, Colli MEA, Elliff FM, Sá Filho MF, Vieira L, Gonzales de Freitas B. Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. *Anim Reprod.* 2017;14:558-571.
Batista EOS, Del Valle TA, Ortolan MDDV, Renno FP, Nogueira GP, Souza AH, Baruselli PS. The effect of circulating progesterone on magnitude of the GnRH induced LH surge: Are there any differences between *Bos indicus* and *Bos taurus* heifers? *Theriogenology* 2017;104:43-48.
Bó GA, Pierson RA, Mapletoft RJ. The effect of estradiol valerate on follicular dynamics and superovulatory response in cows with Syncro Mate B implants. *Theriogenology* 1991;36:169-183.
Bó GA, Adams GP, Pierson RA, Caccia M, Tribulo HE, Mapletoft RJ. Follicular wave dynamics after estradiol-17β treatment of heifers with or without a progestogen implant. *Theriogenology* 1994;41:1555-1569.
Bó GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. *Theriogenology* 1995;43:31-40.
Bó GA, Baruselli PS, Moreno D, Cutaia L, Caccia M, Tribulo R, Tribulo H, Mapletoft RJ. The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology* 2002;57:53-72.
Bó GA, Baruselli PS, Mapletoft RJ. Synchronization techniques to increase the utilization of artificial insemination in beef and dairy cattle. *Anim Reprod*. 2013;10:137-142.
Bó GA, De la Mata JJ, Baruselli PS, Menchaca A. Alternative programs for synchronizing and re-synchronizing ovulation in beef cattle. *Theriogenology* 2016;86:388-396.
Bó GA, Huguenine E, de La Mata JJ, Núñez-Olivera R, Baruselli PS, Menchaca A. Programs for fixed-time artificial insemination in South American beef cattle. *Anim Reprod*. 2018;15(1):952-962.
Bó GA, Baruselli PS, Menchaca A, Mapletoft RJ. Evolution of synchronization protocols and use of fixed-time artificial insemination in beef cattle in South America. *Clinical Theriogenology* 2019a;11:255-263.
Bó GA, Huguenine E, de la Mata JJ, Carneiro RLR, Menchaca A. Pregnancy rates in suckled beef cows synchronized with a shortened progesterone/estradiol-based protocol (J-Synch) and inseminated with conventional or sexed-sorted semen. *Reprod Fertil Dev*. 2019b;31:129 (abstract).
Bó GA, Tschopp JC, Macagno A, Huguenine E, Cedeño AV, de la Mata JJ, Menchaca A. Actualización sobre los protocolos de proestro prolongado con estradiol y GnRH en ganado de carne y leche. *14º Simposio Internacional de Reproducción Animal; 2022;* Carlos Paz, Córdoba, Argentina. Córdoba: *IRAC*; 2022. p.182-199.
Bó GA, Pinargote L, Bernal B, Mendoza B, Ocampo V, Cedeño AV. Follicular characteristics and pregnancy rates in suckling *Bos indicus* cows synchronised with oestradiol-based or gonadotrophin-releasing-hormone-based protocols with a lengthened proestrus. *Reprod Fertil Dev.* 2023;35:220 (abstract).
Bonacker RC, Stoecklein KS, Locke JWC, Ketchum JN, Knickmeyer ER, Spinka CM, Poock SE, Thomas JM. Treatment with prostaglandin F2α and an intravaginal progesterone insert promotes follicular maturity in advance of gonadotropin-releasing hormone among postpartum beef cows. *Theriogenology* 2020a;157:350-359..
Bridges GA, Helser LA, Grum DE, Mussard ML, Gasser CL, Day ML. Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. *Theriogenology* 2008;69:843-851.
Bridges GA, Ahola JK, Brauner C, Cruppe LH, Currin JC, Day ML, Gunn PJ, Jaeger JR, Lake SL, Lamb GC, Marquezini GHL, Peel RK, Radunz AE, Stevenson JS, Whittier WD. Determination of the appropriate delivery of prostaglandin F2α in the five-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. *J Anim Sci*. 2012;90:4814-4822.
Bridges GA, Day ML, Geary TW, Cruppe LH. Deficiencies in the uterine environment and failure to support embryonic development. *J Anim Sci*. 2013;91:3002-3013.
Bridges GA, Mussard ML, Hesler LA, Day ML. Comparison of follicular dynamics and hormone concentrations between the 7-day and 5-day CO-Synch + CIDR program in primiparous beef cows. *Theriogenology* 2014;81:632-638.
Cattaneo L, Prieto C, Ojeda D, Pereira A, Frutos J, Bó GA. The use of a recombinant equine chorionic gonadotropin (reCG) in fixed-time AI programs in beef cattle. *Theriogenology* 2024;227:77-83.
Cedeño A, Maingón R, Gamboa H, Avellán J, Bravo J, Rivera C, Macías I, Guadalupe C, Figueroa V, Bó GA. Protocolos de prolongación de proestro modificados: dinamica folicular en vaquillonas *Bos indicus* de carne en programas de IATF. *13º Simposio Internacional de Reproducción Animal; 2019;*

Córdoba, Argentina. Córdoba*: IRAC*; 2019. p.283 (abstract).
Cedeño AV, Cuervo R, Tríbulo A, Tríbulo R, Andrada S, Mapletoft RJ, Menchaca A, Bó GA. Effect of expression of estrus and treatment with GnRH on pregnancies per AI in beef cattle synchronized with an estradiol/progesterone-based protocol. *Theriogenology* 2021a;161:294-300.
Cedeño AV, Bó GA. Effect of length of insertion of a progesterone device on follicular diameter, time of ovulation and pregnancy rates in *Bos indicus* cows treated with an estradiol/progesterone-based protocol with a prolonged proestrus. *Reprod Fertil Dev*. 2021b;33:166-167 (abstract).
Cedeño AV, Pinargote L, Bernal B, Mendoza B, Ocampo V, Bó GA. Tasas de preñez en vaquillonas *Bos indicus* prepúberes sincronizadas con el protocolo J-Synch. *14º Simposio Internacional de Reproducción Animal; 2022;,* Carlos Paz, Córdoba, Argentina. Córdoba: IRAC; 2022. p.297 (abstract).
Colazo MG, Kastelic JP, Mapletoft RJ. Effects of estradiol cypionate (ECP) on ovarian follicular dynamics, synchrony of ovulation, and fertility in CIDR-based, fixed-time AI programs in beef heifers. *Theriogenology* 2003;60:855-865.**Colazo MG, Kastelic JP, Martinez MF, Whittaker PR, Wilde R.** Fertility following fixed-time AI in CIDR treated beef heifers given GnRH or estradiol Cypionate and fed diets supplemented with flax seed or sunflower seed. *Theriogenology* 2004;61:1115-1124.
Colazo MG, Martínez MF, Small JA, Kastelic JP, Burnley CA, Ward D, Mapletoft RJ. Effects of estradiol valerate on ovarian follicle dynamics and superovulatory response in progestin-treated cattle. *Theriogenology* 2005;63:1454-1468.
Cuadro F, García Pintos C, Núñez-Olivera R, Brochado C, Bó GA, Menchaca A. Equine chorionic gonadotropin (eCG) treatment in heifers: double ovulation, twin rate, and pregnancy losses in twin pregnancies. *Theriogenology* 2024;226:213-218.
de Abreu LA, Cutaia L, Perez Wallace S, Santos Resende T, Mancini Carreira AL, de Sa Cunha B, Goncalves de Sousa V, Alves da Silva L, Catussi BLC, Baruselli PS. Efficacy of equine chorionic gonadotrophin-like treatment on follicular dynamics and pregnancy rate in Nelore cows submitted to fixed-time AI. *Reprod Fertil Dev.* 2023;35:222 (abstract).
de la Mata JJ, Bó GA. Sincronización de celos y ovulación utilizando protocolos con benzoato de estradiol y GnRH en períodos reducidos de inserción de un dispositivo con P4 en vaquillonas para carne. *Taurus* 2022;55:17-23.
de La Mata JJ, Núñez-Olivera R, Cuadro F, Bosolasco D, De Brun V, Meikle A, Bó GA, Menchaca A. Effects of extending the length of pro-oestrus in an oestradiol- and progesterone-based oestrus synchronisation program on ovarian function, uterine environment and pregnancy establishment in beef heifers. *Reprod Fertil Dev*. 2018;30:1541-1552.
de la Mata JJ, Morone S, Macagno A, Tschopp JC, Huguenine E, Cedeño A, Bó GA. Tratamiento a base de GnRH y P4 como alternativa para los tratamientos a base de estradiol para la inseminación artificial a tiempo fijo en vacas *Bos taurus* para carne. *Taurus* 2022;93:39-53.
Ferraz Jr MVC, Pires AV, Biehl MV, Santos MH, Barroso JPR, Goncalves JRS, Sartori R, Day ML. Comparison of two timed artificial insemination system schemes to synchronize estrus and ovulation in Nellore cattle. *Theriogenology* 2016;86:1939-1943.
Ferré LB, Jaeschke J, Gatti J, Baladón G, Bellocq E, Fernández G, Rearte R, Kjelland ME, Colazo MG, Thomas JM. Comparison of Gonadotropin Releasing Hormone versus Estrogen-Based Fixed-Time Artificial Insemination Protocols in Grazing *Bos taurus* Suckled Beef Cows. *Animals* 2023;13:2803. https://doi.org/10.3390/ani13172803.
Frutos J. Estrategias Reproductivas durante los programas de IATF. *Séptimo Congreso Internacional de Reproducción Animal*; 2018, 30-31 agosto; Asunción, Paraguay.. CD.
Garcia Pintos C, Cuadro F, Núñez-Olivera R, Brochado C, Fabini F, Abelenda C, Buero J, País V, Caffera C, Menchaca A. Protocolos a base de GnRH en ganado bovino de carne y leche: la experiencia de Uruguay. *14 Simposio Internacional de Reproducción Animal; 2022;* Carlos Paz, Córdoba, Argentina. Córdoba: *IRAC; 2022.* p.141-153.
Geary TW, Whittier WD, Hallford DM, MacNeil MD. Calf removal improves conception rates to the Ovsynch and Co-synch protocols. *J Anim Sci.* 2001a;79:1-4.
Geary WT, Downing ER, Bruemmer EJ, Whittier WD. Ovarian and Estrous Response of Suckled Beef Cows to the Select Synch Estrous Synchronization Protocol. *The Professional Animal Scientist* 2001b;16:1-5..
Gunn PJ, Culp KC, Lemenager RP, Bridges GA. Efficacy of the 5-day CO-Synch ovulation synchronization protocol with or without the inclusion of exogenous progesterone in beef cows. *The Professional Animal Scientist* 2016;32:82-89.
Huguenine E, Peracchia S, Benitez R, Martini H, Cledou G, Bó GA, Callejas S. Effect of the utilization of 5-day CO-Synch protocols combined or not with eCG in suckled cows in postpartum anoestrus. *X*

Symposium on Animal Reproduction; 2013; Córdoba, Argentina. Córdoba: *IRAC*; 2013. p.313 (abstract).
Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. *J Anim Sci.* 2011;79:2253-2259.
Macagno A, de la Mata JJ, Tschopp JC, Bó GA. Pregnancy rates in lactating Holstein cows synchronized with a modified GnRH/progesterone based TAI protocol. *International Congress on Animal Reproduction (ICAR); 2022,* 26-30 june; Bologna, Italy. Bologna: ICAR; 2022. **Macagno AJ, Tschopp JC, de la Mata J, Ezenga A, Bó GA.** Ovulatory follicle size, time of ovulation, and pregnancy rates to AI in lactating dairy cows treated with a new gonadotrophin-releasing-hormone-based protocol with lengthened proestrus. *Reprod Fertil Dev*. 2023;35:219 (abstract).
Macmillan KL, Thatcher WW. Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. *Biol Reprod.* 1991;45:883-889.
Madsen CA, Perry GA, Mogck CL, Daly RF, MacNeil MD, Geary TW. Effects of preovulatory estradiol on embryo survival and pregnancy establishment in beef cows. *Anim Reprod Sci*. 2015;158:96-103.
Madureira G, Consentini C, Motta J, Drum J, Prata A, Monteiro P, Melo LF, Gonçalves J, Wiltbank M, Sartori R. Progesterone-based timed AI protocols for *Bos indicus* cattle II: Reproductive outcomes of either EB or GnRH-type protocol, using or not GnRH at AI. *Theriogenology* 2020;145:86-93.
Marquezini GHL, Mercadante VRG, Olson KC, Jaeger JR, Perry GA, Stevenson JS, Lamb GC. Effects of equine chorionic gonadotropin on follicle development and pregnancy rates in suckled beef cows with or without calf removal. *J Anim Sci.* 2013;91:1216-1224.
Mapletoft RJ, Martinez MF, Colazo MG, Kastelic JP. The use of controlled internal drug release devices for the regulation of bovine reproduction. *J Anim Sci*. 2003;81(2):E28-E36.
Martinez MF, Adams GP, Bergfelt D, Kastelic JP, Mapletoft RJ. Effect of LH or GnRH on the dominant follicle of the first follicular wave in heifers. *Anim Reprod Sci*. 1999;57:23-33.
Martinez MF, Adams GP, Kastelic JP, Bergfelt DR, Mapletoft RJ. Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. *Theriogenology* 2000;15:757-769.
Martinez MF, Kastelic JP, Adams GP, Cook RB, Olson WO, Mapletoft RJ. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. *Theriogenology* 2002;57:1049-1059.
Martínez MF, Kastelic JP, Bó GA, Caccia M, Mapletoft RJ. Effects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. *Anim Reprod Sci.* 2005;86:37-52.
Martínez MF, Kastelic JP, Mapletoft RJ. Effects of estradiol on gonadotrophin release, estrus and ovulation in CIDR-treated beef cattle. *Domest Anim Endo.* 2007;33:77-90.
Menchaca A, Dutra S, Carrau JM, Sapriza F, Bó GA. Improvements of the new J-Synch protocol used for fixed time embryo transfer (FTET) in beef cattle recipients transferred with in vitro produced embryos. *International Congress of Animal Reproduction (ICAR)*; 2016; Tours, France. Tours: ICAR; 2016. p.471 (abstract).**Motta JCL, Colli MHA, Penteado L, Bayeux BM, Mingoti RD, Bó GA, Lugo LC, Rezende RG, Baruselli PS.** Pregnancy rate to FTAI in Nelore and crossbreed heifers submitted to J-Synch protocol (6 days). *Anim Reprod*. 2016;13:401 (abstract).
Núñez-Olivera R, de Castro T, García-Pintos C, Bó GA, Piaggio J, Menchaca A. Ovulatory response and luteal function after eCG administration at the end of a progesterone and estradiol-based treatment in postpartum anestrous beef cattle. *Anim Reprod Sci.* 2014;146:111-116.
Núñez-Olivera R, Cuadro F, Bosolasco D, de Brun V, de la Mata J, Brochado C, Meikle A, Bó GA, Menchaca A. Effect of equine chorionic gonadotropin (eCG) administration and proestrus length on ovarian response, uterine functionality and pregnancy rate in beef heifers inseminated at a fixed-time. *Theriogenology* 2020;151:16-27..
Núñez–Olivera R, Bó GA, Menchaca A. Association between proestrus length, follicular growth, estrous behavior, and pregnancy rate in beef heifers subjected to fixed-time artificial insemination. *Theriogenology* 2022;181:1-7.
Pereira A. Efectos de la IATF en una unidad de cría en la zona norte de Paraguay. *Octavo Congreso Internacional de Reproducción Animal*; 2022, 2-3 junio; Asunción, Paraguay. , 2022. CD.
Pincinato D. Follicular dynamics and fertility in beef suckled cows synchronized with progesterone releasing devices and GnRH. *Master of Science Thesis, Faculty of Agriculture Sciences, National University of Cordoba,* Córdoba, Argentina, 2012.
Pincinato D, Peres LC, Lorentz L, Santana GS, Machado MK, Borges AJ, Lacerda LS, Bó GA. Pregnancy rates in Nelore heifers using a shortened estradiol/progesterone-based protocol that provides for

a lengthened proestrus (J-Synch). *Proceedings of the 32nd Annual Meeting of the Brazilian Embryo Technology Society (SBTE); 2018,* august 16-18; Florianópolis, SC, Brazil. Florianópolis: SBTE; 2018; 15:350 (abstract).
Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology* 1995;44:15-923.
Randi F, Kelly AK, Parr MH, Diskin MG, Lively F, Lonergan P, Kenny DA. Effect of ovulation synchronization program and season on pregnancy to timed artificial insemination in suckled beef cows. *Theriogenology* 2021;172:223-229.
Revah I, Butler WR. Prolonged dominance of follicles and reduced viability of bovine oocytes. *J Reprod Fertil*. 1996;106:39-47.
Sá Filho MF, Santos JEP, Ferreira RM, Sales JNS, Baruselli PS. Importance of estrus on pregnancy per insemination in suckled *Bos indicus* cows submitted to estradiol/progesterone-based timed insemination protocols. *Theriogenology* 2011;76:455-463.
Sales JNS, Carvalho JBP, Crepaldi GA, Cipriano RS, Jacomini JO, Maio JRG, Souza JC, Nogueira GP, Baruselli PS. Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in *Bos indicus* cows submitted to a timed artificial insemination protocol. *Theriogenology* 2012;78:510–516.
Savio JD, Thatcher WW, Morris GR, Entwistle K, Drost M, Mattiacci MR. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J Reprod Fertil*. 1993;98:77-84.
Ré MG, Racca G, Filippi L, Veneranda G, Bó GA. Sincronización de la ovulación y tasas de preñez en vaquillonas lecheras tratadas con protocolos que prolongan el proestro. *Taurus* 2021;91:28-45.
Small JA, Colazo MG, Kastelic JP, Mapletoft RJ. Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. *Theriogenology* 2009;71:698-706.
Souto LA, Maquivar M, Mussard ML, Bridges GA, Grum DE, Day ML. Fertility and luteal regression with 5-d CIDR synchronization programs in postpartum beef cows using differing luteolytic treatments. *J Anim Sci*. 2009;87(2):372 (abstract).
Uslenghi G, González Chavez S, Cabodevila J, Callejas S. Effect of estradiol cypionate and amount of progesterone in the intravaginal device on synchronization of estrus, ovulation and on pregnancy rate in beef cows treated with FTAI based protocols. *Anim Reprod Sci.* 2014;145:1-7.
Villarraza CJ, Antuña S, Tardivo MB, Rodríguez MC, Mussio P, Cattaneo L, Fontana D, Díaz PU, Ortega HH, Tríbulo A, Macagno A, Bó GA, Ceaglio N, Prieto C. Development of a suitable manufacturing process for production of a bioactive recombinant equine chorionic gonadotropin (reCG) in CHO-K1 cells. *Theriogenology* 2021;172:223-229.
Whittier WD, Currin JF, Schramm H, Holland S, Kasimanickam RK. Fertility in Angus cross beef cows following 5-day CO-Synch + CIDR or 7-day CO-Synch + CIDR estrus synchronization and timed artificial insemination. *Theriogenology* 2013;80:963-969.
Wiltbank JN, Zimmerman DR, Ingalls JE, Rowden WW. Use of progestational compounds alone or in combination with estrogen for synchronization of estrus. *J Anim Sci*. 1965;24:990-994.