Synchronization of estrus and ovulation in bovine females.
Endocrine bases and treatments used

Sincronización del estro y ovulación en hembras bovinas de razas cárnicas. Bases endocrinas y protocolos usados

Hernández-Coronado Cyndi1ID, Rosales-Torres Ana1ID, Vázquez-López Salomón2ID, Guzmán-Sánchez Adrián*1ID

1Universidad Autónoma Metropolitana, Unidad Xochimilco, Departamento Producción Agrícola y Animal, Calzada del Hueso 1100, Coyocán, CDMX 04960 México. 2Universidad Autónoma Metropolitana, Unidad Xochimilco. Maestría en Ciencias Agropecuarias. Calzada del Hueso 1100, Coyocán, CDMX 04960 México. *Author for correspondence: Adrián Guzmán-Sánchez. Email: cyndibash87@gmail.com, anamexico@gmail.com, mvzsalovazquez@gmail.com, aguzman@correo.xoc.uam.mx

Abstract
In Mexico, reproductive efficiency of cows in calf production systems is low and the use of estrus and ovulation synchronization schemes can contribute to improve it. The objective of this review was to describe the endocrine regulation of the estrous cycle and follicular dynamics in cattle, highlighting how, using exogenous hormones, these processes can be manipulated to implement different estrous and ovulation synchronization protocols. In addition, results of pregnancy rate at synchronized estrus (PRSE) with the different estrous and ovulation synchronization protocols reported in the literature are presented. Reviews and original articles on topics of estrous cycle and follicular development in cattle were collected and PRSE data were obtained from scientific articles published between 2000 and 2023. The reported PRSE ranges from 23 to 76 %, however, although the range is wide, in estradiol and GnRH based protocols about 50 % of the PRSE data collected are between 45 and 55 %. In progesterone-based protocols, 50 % of the data report PRSE between 55 and 65 %. Currently, there are three main estrous and ovulation synchronization protocols with which, according to the literature reviewed, PRSE between 45 and 65 % can be obtained in most occasions.

Keywords: oestrus cycle, waves of follicular growth, synchronization of estrus and ovulation, beef cows.

Resumen
En México, la eficiencia reproductiva de las vacas en los sistemas de producción de becerro es baja y el uso de esquemas de sincronización del estro y la ovulación puede contribuir a mejorarla. El objetivo de esta revisión fue describir la regulación endocrina del ciclo estral y la dinámica folicular en los bovinos, resaltando cómo, a través del uso de hormonas exógenas se pueden manipular estos procesos para implementar los diferentes protocolos de sincronización del estro y la ovulación. Además, se presentan resultados de tasa de preñez a estro sincronizado (TPES) con los diferentes protocolos de sincronización de estro y ovulación que se reportan en la bibliografía. Se recopilaron revisiones y artículos originales sobre temas de ciclo estral y desarrollo folicular en bovinos y se obtuvieron datos de TPES de artículos científicos publicados entre 2000 y 2023. La TPES reportada oscila entre 23 hasta 76 %, sin embargo, aunque el rango es amplio, en protocolos basados en estradiol y GnRH alrededor del 50 % de los datos de TPES...
colectados están entre 45 y 55 %. En protocolos basados en progesterona el 50 % de los datos reportan TPES de entre 55 y 65 %. En la actualidad, existen tres principales protocolos de sincronización de estro y ovulación con los que, según la bibliografía revisada, se puede obtener TPES de entre 45 y 65% en la mayoría de las ocasiones. **Palabras clave:** ciclo estral, olas de crecimiento folicular, sincronización de estro y ovulación, vacas productoras de carne.

**INTRODUCTION**

Rearing calves for beef production depends largely on the reproductive efficiency of dams (Alvarez et al., 2018). In Mexico, in general and specifically in the tropical regions of the country, reproductive efficiency in calf production systems is low (Lassala et al., 2020). It has been reported that only 32.6 % of cows in the national herd are pregnant, cows in the Northeast and Central zone have the highest gestation percentage (41 %), while in the Northern region the gestation percentage is only 25 % (Gutiérrez, 2018). Although there are factors such as breed, postpartum anestrus, lactation and nutritional status affecting reproductive efficiency in these production systems, there are also some reproductive management aspects that can exacerbate this problem. In Mexico, about 90 % of the country’s calf producers use continuous mating, only between 2.4 and 9.4 % use artificial insemination (AI) and less than 10 % perform estrus synchronization (Lassala et al., 2020).

Knowledge of the endocrine control of the estrous cycle and the follicular dynamics physiology in cows has allowed the development of biotechnologies such as estrus and ovulation synchronization that in turn facilitate and make AI more efficient in beef cattle (Marizancén & Artunduaga, 2017). These biotechnologies applied correctly improve the reproductive efficiency of cattle (Colazo et al., 2018; Barusseli et al., 2018). In postpartum Nelore cows, the use of an estrus synchronization protocol prior to the onset of mating increased 1.5 times the probability of cows becoming pregnant during this period, as well as the pregnancy rate of the mating (52.2 vs. 27.6 %; Ferreira et al., 2018). In beef cows, the use of estrus and ovulation synchronization protocols at the beginning of the mating, allows inducing and uniformizing the manifestation of estrus in a pre-established period of time of short duration, which facilitates performing AI, scheduling calving times and inducing ovarian activity in heifers and cows in anestrus (Colazo & Mapletoft, 2014; Baruselli et al., 2018).

Estrus and ovulation synchronization protocols are based on the use of hormones to manipulate follicular dynamics and the duration of the luteal phase of the estrous cycle (Lamb et al., 2010; Colazo & Mapletoft, 2014) so it is important to understand these processes in detail. Hormones used to manipulate follicular dynamics are gonadotropin-releasing hormone (GnRH), estrogens and progesterone. To manipulate the duration of the luteal phase, prostaglandin F2α (PGF2-α) and progesterone can be used (Lamb et al., 2010; Bó & Baruselli, 2014; Colazo & Mapletoft, 2014; Bó et al., 2016).
It is important when applying hormonal treatments to manipulate the reproductive response of cattle to know the mechanism by which physiological and endocrine changes occur at each stage of the estrous cycle to be modified, as well as effects that each hormone has in a given protocol. This paper aims to describe the endocrine regulation of the estrous cycle and follicular dynamics in cattle, highlighting how, using exogenous hormones, and these processes can be manipulated to implement different estrus and ovulation synchronization protocols. In addition, results of pregnancy rate at synchronized estrus (PRSE) with different estrus and ovulation synchronization protocols reported in the literature are presented.

MATERIAL AND METHODS

We searched Pubmed, ScienceDirect, Google Scholar and SciELO databases using the keywords "Bovine estrus cycle" and "Bovine follicular waves" and selected the most important reviews and original articles on these topics, including those published by our group. To describe the synchronized estrus pregnancy rate (PRSE) results obtained with the main protocols currently in use, a search only in Pubmed was performed using the keywords "estrus synchronization beef cows estradiol" to obtain data reported using estradiol-based protocols and with the keywords "estrus synchronization beef cows GnRH", articles with the use of GnRH-based protocols were retrieved. In addition to the articles we retrieved in the latter search, we selected those that used progestogen-based protocols. Publications from 2000 to 2023 were selected in both searches. The main selection criteria for PRSE data were those where treatment assignment was completely randomized. Additionally, data were taken from experimental groups that evaluated the effect of equine chorionic gonadotropin (eCG) use, estrus presentation effect, cyclicity and temporary weaning. When in an article they evaluated variables other than those mentioned above, only the data from the control group were taken. With the PRSE data from each experimental group, frequency histograms or scatter plots were made to describe the variation of the data.

Endocrine Control of the Estrous Cycle in Cattle

Female domestic mammals from puberty and throughout their reproductive life will periodically present estrous cycles, which are defined as the period from the onset of one estrus to the onset of the next (Lamb & Mercadante, 2016). The estrous cycle is characterized by a series of anatomical, endocrine and behavioral changes aimed at ovulation and preparing the uterine environment for possible gestation (Bó et al., 2003). In cattle, each estrous cycle has an average duration of 20 days in heifers and 21 days in cows (Sartori & Barros, 2011). The estrous cycle is composed of four stages: estrus, metaestrus, diestrous, proestrus and two phases: the follicular, estrogenic or proliferative phase, which includes proestrus and estrus, and the luteal, progesterational or secretory
phase, which includes metaestrus and diestrous (Figure 1). In the follicular phase the ovulatory follicle develops, so the dominant hormone is estradiol. This steroid promotes, at the uterine level, the proliferation of endometrial cells. In the luteal phase, the development and functionality of the CL occurs, so the dominant hormone is progesterone that stimulates the endometrium to secrete uterine milk or histone (Rathbone et al., 2001; Sartori & Barros, 2011).

The estrous cycle establishment and the changes that occur in it are regulated by the synthesis and secretion of hormones from the hypothalamus. GnRH, from the pituitary gland produces luteinizing hormone and follicle stimulating hormone (LH and FSH), from the follicle that secretes estrogen and inhibin, from the LC that produces progesterone and oxytocin, and from the endometrium that releases PGF2α (Sartori & Barros, 2011).

During proestrus, FSH production remains low and constant because it is not regulated by GnRH, while LH begins to increase its frequency of secretion and decrease the amplitude of its pulses. This promotes the final maturation of the follicle by increasing the synthesis of estradiol and inhibin (Rathbone et al., 2001). The increase of these two hormones exerts a negative feedback on FSH synthesis at the pituitary level (Aerts & Bols, 2010). On the other hand, towards the end of proestrus and beginning of estrus, estradiol exerts a positive feedback effect on GnRH by acting on α and β receptors located on glutamatergic and kispeptinergic neurons (Kenealy & Terasawa, 2012). This estradiol effect triggers the preovulatory GnRH-LH peak, and with it ovulation (Garverick & Smith, 1993; Forde et al., 2011; De Graaff & Grimard, 2018). To trigger ovulation, which occurs between 10 to 12 hours after the end of estrus, LH promotes the synthesis of prostaglandins and enzymes to facilitate the rupture of follicular walls. In addition, LH stimulates the restart of meiosis in the oocyte (Delgado et al., 2011). After ovulation, the CL develops from the ovulated follicle by the effect of LH and progesterone concentrations gradually increase to reach their maximum concentration at the end of metaestrus and beginning of diestrous (Forde et al., 2011). Peak progesterone concentrations are kept constant throughout the entire estrus to prevent ovulation of dominant follicles developed during the luteal phase of the cycle (Baruselli et al., 2007).

If the ovulated oocyte is not fertilized, around days 16 and 17 of the estrous cycle, the endometrium will secrete PGF2α to induce CL regression (Rosales-Torres & Guzmán, 2011; Sartori & Barros, 2011). For this, PGF2α by binding to its receptor on CL blood vessels causes an elevation of intracellular Ca++ to induce vasoconstriction. Vasoconstriction reduces the supply of nutrients, oxygen and cholesterol to the LC, causing a reduction in progesterone synthesis and apoptosis of the luteal cells (Rosales-Torres & Guzmán, 2008; Shirasuna et al., 2012). Luteolysis causes a reduction in the serum concentration of progesterone so that the negative feedback exerted by progesterone on GnRH disappears (Rathbone et al., 2001).
Waves of Follicular Growth

Follicular development during the estrous cycle in cattle occurs in waves and it is a highly selective process where usually only one follicle can ovulate and the fate of the rest of the follicles in the same cohort is atresia (Rosales-Torres et al., 2012). In each wave of follicular growth, a dominant follicle develops, although only the dominant follicle of the last wave will ovulate (Aerts & Bols, 2010; Bó et al., 2016; Ginther, 2016). Follicular growth waves are composed of three phases: cyclic recruitment, selection and dominance (Aerts & Bols, 2010; Fortune et al., 1991; Rosales-Torres et al., 2012).

During cyclic recruitment, a cohort of small antral follicles initiate growth in response to a transient FSH spike (Mihm et al., 2000; Driancourt, 2001; Aerts & Bols, 2010). This phase lasts about 2 days during which FSH stimulates follicle growth (Webb et al., 2004; Rodgers & Rodgers, 2010). Selection occurs at the end of the common growth period, when one of the recruited follicles, is selected as dominant to continue its growth (Sirard, 2016; Rosales-Torres et al., 2012). Finally, dominance refers to the mechanism by which the follicle selected as dominant has a rapid development and suppresses the growth of subordinate follicles Ginther et al., 1989; Webb et al., 2004). To this end, estradiol and the inhibin produced in it prevent the synthesis and secretion of FSH (Beg & Ginther, 2006). This reduction of FSH causes atresia of the subordinate follicles, while the dominant follicle continues its growth at a rate of up to 1.6 mm per day (Sirois & Fortune 1988; Rosales-Torres et al., 2012). If the dominant follicle develops during the luteal phase of the estrous cycle, circulating concentrations of progesterone prevent the preovulatory LH peak so it will be unable to ovulate and will become atretic (Figure 1). In the absence of a functional CL, the dominant follicle by its high estradiol production induces the preovulatory LH peak and thus ovulation (Driancourt, 2001; Moore & Thatcher, 2006).
Figure 1. Schematic representation of the stages and phases of the estrous cycle in cattle and of the follicular development waves

It is important to note that as long as the dominant follicle is estrogensively active, a new wave of follicular growth cannot emerge (Webb et al., 2004; Rosales-Torres et al., 2012). However, if the dominant follicle ovulates or becomes atretic, the blockade on FSH will disappear as estradiol and inhibin are no longer produced and a new transient elevation of this hormone will occur, which will stimulate the emergence of a new wave of follicular growth (Sartori et al., 2004; Beg & Ginther, 2006).

There are some species and breed differences in follicular dynamics (Baruselli et al., 2007). In Bos taurus cattle, the selection of the dominant follicle occurs 2 days after the initiation of the follicular wave and generally the follicle that reaches 8.5 mm in diameter will be the one selected as dominant (Fortune et al., 1991; Fortune et al., 2001). In the case of Bos indicus cows, the selection of the dominant follicle will be 2.6 days after the start of the wave and the follicle with a diameter of approximately 5.9 ± 0.4 mm will be selected (Sartori & Barros, 2011). Baruselli et al. (2018) report that Brahman, Nelore and Gyr cows can present between two and four waves of follicular development per estrous cycle. While in Bos taurus cows two to three waves predominate (Sartori et al., 2004). In addition to the difference in the number of follicular waves, Bos indicus females recruit a greater number of follicles per follicular growth wave than Bos taurus females (33.4±3.2
versus 25.4±2.5 respectively). Finally, in *Bos taurus* females with two follicular growth waves the largest diameter of the dominant follicle is 17.1 mm for the first wave and 16.5 mm for the second wave (Ginther, 2016). In *Bos indicus* the largest diameters of the dominant follicle are 11.3 mm, 12.1 mm and 10.4 mm for the first, second and third growth waves respectively (Figueiredo *et al.*, 1997).

**Oestrus and Ovulation Synchronization**

Oestrus and ovulation synchronization protocols have important advantages for the reproductive management of beef cattle and have evolved over time to be more effective. The implementation of these protocols facilitates the use of fixed-time artificial insemination (FTAI) or estrus detected, homogenizes calf birth, allows calving scheduling, newborn calf care, and increases pregnancy rates (Abel *et al.*, 2017). Currently, hormonal manipulation of the estrous cycle in cattle to synchronize estrus and ovulation is based on four major principles;

1) **Simulate the luteal phase of the estrous cycle.** This is achieved by exogenous administration of progesterone using intravaginal devices that release progesterone (DIP) constantly (Colazo & Mapletoft, 2014) or by using oral progestogens such as melengestrol acetate (MGA; Lamb *et al.*, 2010). DIPs are currently the most commonly used and are recommended for use for periods of 5 to 7 days (Abel *et al.*, 2017; Williams & Stanko, 2020). The physiological rationale for the use of DIPs is that, upon withdrawal of treatment, blood progesterone concentrations are rapidly reduced. This causes animals that do not have a corpus luteum to go into proestrus and maturation of a dominant follicle occurs that produces sufficient estradiol to increase the frequency of pituitary LH pulses and the likelihood of ovulation (Rathbone *et al.*, 2001; Lamb *et al.*, 2010; Sartori & Barros, 2011).

2) **Accelerate CL regression by administration of PGF2α or its synthetic analogues.** The application of PGF2α induces lysis of the CL to cause reduction in endogenous progesterone concentrations and induce a new follicular phase in animals that have a functional CL (Abreu *et al.*, 2018). PGF2α induces lysis of the CL when applied at least 6 to 7 days after the onset of estrus. Since prior to this time, the CL is in development and there are many survival factors in the surrounding environment such as LH, VEGF and IGF-I that prevent the luteolytic effect of PGF2α (Wenzinger & Bleul, 2012; Abel *et al.*, 2017; Scarpa *et al.*, 2019). It is important to note that in the absence of a CL PGF2α has no effect, however for management issues and the need to have experience to palpate the presence of a CL, PGF2α is usually applied even when there is no in CL in ovary.

3) **Synchronize the onset of a new wave of follicular growth.** This is achieved by the application of GnRH or estradiol. When GnRH or its analogues are applied, an LH peak is induced and if the female has a dominant follicle, it will ovulate. Ovulation of the dominant follicle causes a reduction of estradiol and inhibin, which allows an elevation of FSH to initiate a new wave of follicular growth (Adams *et al.*, 1992; Aerts & Bols, 2010). As for the use of estradiol or its analogues, they are applied in high doses (2 mg), to induce a negative feedback on FSH at the pituitary level and thereby cause atresia of the follicles of the growth wave in turn present in the ovary. When the follicles become atretic, they stop secreting estradiol, inhibin, the negative feedback they exerted on FSH is
eliminated, and the concentrations of this gonadotropin are elevated to initiate a new wave of follicular growth (Bó et al., 1994; Scarpa et al., 2019).

4) **Induce ovulation of the dominant follicle of the synchronized growth wave.** In beef cattle, ovulation can be induced by application of GnRH at the time of artificial insemination or with low-dose (1 ng) application of estradiol in proestrus animals to induce estrous behavior and the preovulatory LH peak (Bó et al., 2016).

**Main Protocols Currently Used**

To date, the most commonly used treatments for estrus and ovulation synchronization are based on DIP’s. The protocols differ in the hormone used to synchronize follicular development, which can be GnRH, synthetic estrogens or progestogens. Thus, the estrous and ovulation synchronization protocols currently used are based on estradiol, GnRH or progesterone (Figure 2), although most protocols use a combination of these hormones (Bó & Baruselli, 2014; Lamb & Mercadante, 2016; Bó et al., 2016).

**Estradiol-based protocols**

Estradiol-based treatments (Figure 2A to 2C) consist of the insertion of a DIP plus an injection of 2 mg estradiol benzoate (BE) intramuscularly on day 0. Estradiol allows for the induction of the onset of the follicular growth wave and thus ensures the presence of an estrogenically active follicle containing a viable oocyte at the time of AI (Bó et al., 1994; Uslenghi et al., 2014; Bó et al., 2016). The DIP is removed 7, 8 or 9 days after insertion and PGF2α is applied to lyse a possible CL. This ensures the reduction of progesterone in blood and the onset of the follicular phase. After this, ovulation of the dominant follicle of the synchronized wave should be induced, for which one can proceed in several ways in relation to DIP withdrawal: 1) apply 1 mg of BE 24 hours later, 2) apply GnRH 54 hours later or 3) apply estradiol cypionate (ECP; 0.5 or 1 mg) upon DIP withdrawal. With the use of these protocols, it is recommended to perform IATF between 54 and 64 hours after DIP withdrawal (Colazo et al., 2003; Sales et al., 2012).

**PRSE in heifers treated with estradiol-based protocols**

Using estradiol-based protocols in heifers, the lowest PRSE reported in the articles consulted was 39 % (Reineri et al., 2023) while the highest was 59 % (Silva et al., 2018a). Regarding differences by ovulation inducer, PRSE did not differ statistically in heifers with the use of GnRH (59 %; Silva et al., 2018b) or DBS (53 %; Silva et al., 2018b) or between the use of BE (48 %) and DBS (47 %; Pfeifer et al., 2014).
Figure 2. Main estrous and ovulation synchronization protocols currently used in beef cattle. A-C) Estradiol-based protocols using BE (A), GnRH (B) or ECP (C) as ovulation inducers. D-F) GnRH-based protocols without (D) or with the use of a progesterone-releasing intravaginal device (DIP) for seven (E) or five (F) days. G and H) Progesterone-based protocols without the use of GnRH prior to PGF2α application (G) or with the use of GnRH seven days prior to PGF2α application (H).

The use of eCG at the time of DIP withdrawal in heifers has not been as extensively evaluated as in cows (see below), however, the use of 200, 300 or 400 IU of eCG does
not seem to modify PRSE (Pinto et al., 2020). From the data collected in heifers, in no case are differences in PRSE between animals in estrus or pubertal status reported. However, the presence of CL at the beginning of treatment indicates that heifers have started to cycle. In this regard, Silva et al. (2018b) report that PRSE is similar in heifers without the presence of CL at the start of treatment when using GnRH (50.5 %) or ECP (50.5 %) as ovulation inducers. However, PRSE was higher in animals with CL when GnRH (68 %) was used to induce ovulation than when DBS (55 %) was used. This evidence suggests that, in heifers treated with estradiol-based protocols, GnRH, BE or DBS can be used interchangeably as ovulation inducers. However, if cycling heifers can be detected at the start of treatment, it is better to use GnRH to induce ovulation in these heifers.

**PRSE in cows treated with estradiol-based protocols**

In lactating cows, the lowest reported PRSE was 23 % (Malik et al., 2012) and the highest was 72 % (Rodrigues et al., 2018). In this group of animals treated with estradiol-based protocols most of the PRSE data (73%) reported in the articles consulted was between 41 and 60 % (Figure 3A). For non-lactating cow data, the lowest PRSE was 44 % and the highest was 62 % (Uslenghi et al., 2014).

As an ovulation inducer, in estradiol-based protocols, range PRSE reported in lactating cows with the use of from 23 to 58 %, whereas when DBS was used as an ovulation inducer, 88 % of the reported PRSE data ranged from 41 to 70 % (Figure 3B). These data suggest that PRSE may be increased if DBS is used compared to BE as ovulation inducers. However, in articles where different ovulation inducers were compared in lactating cows treated with estradiol-based protocols, no differences were observed between the use of BE or ECP (Sales et al., 2012; Uslenghi et al., 2014; Uslenghi et al., 2016).

The use of eCG (300 IU) at the time of DIP withdrawal is a strategy to increase PRSE in lactating cows treated with estradiol-based protocols. As we can see in Figure 3C, PRSE reported when eCG was not used, in 100 % of the data retrieved from the literature, was less than 61 %, whereas when eCG was used 100 % of the PRSE data were between 45 and 72 %. These reports suggest that PRSE in lactating cows treated with estradiol-based protocols can be improved with the use of eCG. This hypothesis was confirmed by Pessoa et al. (2016) who report that the use of eCG at the time of DIP withdrawal increases PRSE (45%) compared to cows not given eCG (30 %). ECG acts primarily on FSH receptors in the follicle to promote estradiol synthesis and follicular growth (Murphy & Martinuk, 1991). It has been reported that increased follicular development can improve oocyte quality and thus fertility (Simões et al., 2018) which explains why, the use of this hormone improves PRSE in estradiol-based protocols.
Figure 3. Pregnancy rate to synchronized estrus (PRSE) with estradiol-based protocols in lactating cows. A) Distribution of total PRSE data. B) Distribution of PRSE data with the use of estradiol benzoate (BE) and estradiol cypionate (ECP) as ovulation inducers. C) Distribution of PRSE data with or without the use of 300 IU of equine chorionic gonadotropin (eCG) at the time of DIP withdrawal. Information compiled from: Ross et al., 2004; Sa Filho et al., 2010; Malik et al., 2012; Campos et al., 2013; Pfeifer et al., 2015; Uslenghi et al., 2016; Pessoa et al., 2016; Cooke et al., 2016; Rodrigues et al., 2018; Santos et al., 2018; Silva et al., 2018a, Crepaldi et al., 2019; Oliveira-Filho et al., 2020; Noronha et al., 2020; Diniz et al., 2021; Alves et al., 2021; Pfeifer et al., 2022; Barbosa et al., 2022; Rodriguez et al., 2023; Aragunde-Vieytes et al., 2023

In lactating beef cows, the presence of calf reduces GnRH/LH secretion (Crowe, 2008), so temporary weaning is recommended from DIP removal until FTAI (Crowe, 2008). However, Cooke et al. (2016) report that PRSE is similar in cows. Temporary weaning was performed (45.6 %) than in cows where it was not performed (46.6 %). Pfeifer et al., (2014) report a PRSE of 58.1 % with temporary weaning, which is within the range of PRSE reported in the rest of the articles reviewed where temporary weaning is not reported to have been performed (Malik et al., 2012; Rodrigues et al., 2018). This evidence suggests that temporary weaning is not necessary and that estradiol-based protocols can counteract the negative effect of calf presence on GnRH/LH secretion.

Another factor that may affect PRSE is the presentation of estrus after DIP withdrawal. PRSE was higher (P<0.05) in lactating and non-lactating cows that presented estrus, than in those that did not present estrus (Cedeño et al., 2021; Pessoa et al., 2016; Rodrigues et al., 2018). The lack of estrous behavior may be indicative of the absence of a mature preovulatory follicle capable of producing sufficient estradiol to stimulate estrus and ovulation, which would explain why animals that do not present estrus have a lower PRSE.

Recently, a variation on estradiol-based protocols has been reported where DIP is used for only 5 or 6 days. This protocol has been termed J-synch and in beef heifers treated with this protocol there is a higher PRSE (61.9 %) compared to heifers treated with the conventional protocol (51.4 %; Bó et al., 2016). Similar to what was observed in heifers
treated with the conventional estradiol-based protocol, heifers treated with J-synch cycling have a higher PRSE than heifers in anestrus (Núñez-Olivera et al., 2022; Zwiefelhofer et al., 2021). Likewise, in lactating cows, the use of J-synch tends to increase PRSE (74.1 %) when compared to the GnRH-based protocol (66.5 %; Macmillan et al., 2020). Additionally, in this type of protocols, the application of eCG at the time of DIP withdrawal increases PRSE (Núñez-Olivera et al., 2020). The reduction from 8 to 5 days in the use of DIP in estradiol-based protocols avoids the presence of persistent follicles since the release of progesterone by DIP is sufficient to exert negative feedback for GnRH, which favors follicular turnover (Day, 2015).

**GnRH-based protocols**

GnRH-based treatments consist of the administration of this hormone on day zero to induce ovulation of the dominant follicle and thereby promote the initiation of a new wave of follicular growth. Subsequently, on day seven, PGF2α is applied to induce regression of the CL and 48 to 72 hours later a second dose of GnRH is applied (Figure 2D) to induce ovulation and TFAI is performed (Martínez et al., 1999; Martínez et al., 2000). The effectiveness of this treatment depends largely on whether the animals ovulate after the first application of GnRH. If animals ovulate, a CL will form, so by applying PGF2α on day 7, serum progesterone concentrations will be reduced, animals will enter a follicular phase and there will be a pre-ovulatory follicle that will ovulate with the second application of GnRH (Bó et al., 2016; Ginther, 2016). Because the percentage of ovulation after the first application of GnRH is highly variable (Bó et al., 2016), this protocol has been supplemented with the use of a DIP along with the first application of GnRH, which must be removed at the time of PGF2α application (Figure 2E). Thus, regardless of whether animals ovulate after the first application of GnRH, when the DIP is removed, all will be in proestrus. Additionally, this protocol has been shortened, using DIP for only 5 days and applying two injections of PGF2α, one at the time of DIP withdrawal and the second 6 to 8 hours later (Figure 2F).

**PRSE in heifers treated with GnRH-based protocols**

In heifers treated with GnRH-based protocols using DIP for 5 days, most PRSE data (91%) were between 51-70 %. In contrast, when DIP was used for 7 days, most PRSE data were between 41-60 % (Figure 4A). These data suggest that PRSE may be increased if DIP is used for 5 days compared to 7 days.

When DIP is used for 5 days in GnRH-based protocols, 2 injections of PGF2α are recommended at the time of DIP withdrawal because ovulating animals, with the first application of GnRH, have a young CL that are under the influence of trophic stimuli that promote their development (Niswender et al., 2000). According to the information consulted, PRSE is reduced in heifers treated with protocols based on GnRH+DIP for 5
days, if a single injection of PGF2α is used compared to the use of two injections of this hormone (Kasimanickam et al., 2012; Kasimanickam et al., 2014; White et al., 2016; Helguera et al., 2018). For this reason, it is most advisable to give two injections of PGF2α when deciding to use DIP for 5 days.

Estrous presentation prior to FTAI has shown contrasting results on PRSE in heifers treated with GnRH-based protocols. Heifers synchronized with the GnRH protocol and DIP for 7 days that present estrus have similar PRSE to heifers that did not present estrus (Martínez et al., 2002; Knickmeyer et al., 2019). On the other hand, Oosthuizen et al. (2018a) and Speckhart et al. (2022), using the same protocol, report that PRSE is higher in heifers that present estrus prior to insemination than those that do not. Thus, it appears that PRSE in heifers treated with GnRH-based protocols may be partially dependent on estrus presentation. However, inseminating only heifers in estrus, in addition to presenting a management challenge to detect estrus, may reduce the calving rate at the end of mating, so it is recommended to inseminate all animals regardless of whether they are in estrus or not.

Regarding pubertal status at the start of treatment, it appears that PRSE does not differ between pubertal and peri-pubertal animals subjected to GnRH-based protocols with use of DIP for 7 or 5 days (Busch et al., 2007; Helguera et al., 2018; Knickmeyer et al., 2019). The use of GnRH and DIP in these protocols, besides synchronizing estrus and ovulation, can induce ovarian activity in peri-pubertal heifers with good body development (Colazo & Mapletoft, 2014; Baruselli et al., 2018), explaining why, there are no differences in PRSE between pubertal and peri-pubertal heifers.

The time between DIP withdrawal and AI has shown contradictory effects on PRSE. Helguera et al., (2018) report no difference in PRSE between heifers treated with GnRH and DIP for 5 days and inseminated at 66 hours after DIP withdrawal with heifers inseminated at 72 hours. In contrast, in heifers treated with the same protocol, PRSE is higher when insemination was performed at 54 hours after DIP withdrawal than when insemination was performed at 72 hours (Kasimanickam et al., 2012). Although the differences in these results may be due to many factors, it is important to note that the longer the time between DIP withdrawal and AI, the risk that the dominant follicle will ovulate, compromising oocyte viability and fertility.

**PRSE in lactating cows treated with GnRH-based protocols**

The distribution of PRSE data in lactating cows treated with GnRH-based protocols without the use of DIP or with its use for 5 or 7 days is shown in Figure 4B. The range of PRSE in lactating cows treated with GnRH-based protocols without the use of DIP was from 31 % (Stevenson et al., 2003) to 64 % (Small et al., 2009). While in females subjected to GnRH-based protocols and DIP for 5 days, the range was 33% (Williams & Stanko,
to 69% (Kasimanickam et al., 2009) and animals where the GnRH-based protocol was used, but with DIP for 7 days, the range was 38% (Rosales-Torres et al., 2017) to 71% (Lamb et al., 2001).

PRSE is higher in animals in which DIP was used for 7 days after the first application of GnRH compared to those without (Lamb et al., 2001 and Larson et al., 2006). Regarding differences between using DIP for 7 or 5 days, the PRSE data collected suggest no difference (Figure 4B), however, it has been reported that PRSE is higher when using DIP for 5 days compared to 7 days (Whittier et al., 2013). Based on these data, the use of DIP for either 5 or 7 days is recommended in cows treated with GnRH-based protocols to ensure that animals that do not ovulate with the first application of GnRH enter the follicular phase at the time of DIP withdrawal (Bó et al., 2016; Ginther, 2016). This increases the number of animals responding to treatment and with it the PRSE.

As mentioned above, the time between DIP removal and AI can vary between 48 and 72 hours. From the PRSE data we collected from lactating cows treated with GnRH+DIP-based protocols for 5 days, in the majority (85%) FTAI was performed within 72 hours of DIP withdrawal, the lowest PRSE was 33% (Williams & Stanko, 2020) and the highest was 69% (Kasimanickam et al., 2009). In lactating cows treated with the GnRH-based protocol with and without the use of DIP for 7 days, the time between PGF2-α application and AI as well as the reported PRSE results were highly variable (Figure 4C and 4D). In lactating cows in which DIP was used for 7 days, the highest PRSE (71%) was reported when FTAI was performed at 48 (Lamb et al., 2001) and 72 hours (Nash et al., 2012), while the lowest (38%) was when AI was performed at 72 hours (Rosales-Torres et al., 2017). Busch et al. (2008), show that PRSE is higher when AI is done at 66 h than when it is done at 54 h after DIP withdrawal. However, due to the variation in PRSE shown in Figure 4C the effect of time between DIP withdrawal and AI in 7-day GnRH and DIP-based protocols may not be significant. Finally, in lactating cows treated with GnRH-based protocols without the use of DIP, PRSE ranged from 31% when FTAI was performed at 48 hours after PGF2α application (Stevenson et al., 2003) to 64% reported when FTAI was performed at 64 hours after PGF2α application (Small et al., 2009).

Pre-AI estrus presentation, ovarian status at baseline, eCG use, and temporary weaning can affect PRSE in lactating cows treated with GnRH-based protocols. Several reports show the effect of presenting estrus prior to AI on PRSE (Nash et al., 2012; Thomas et al., 2014; Hill et al., 2016a; Hill et al., 2016b; Abel et al., 2017) and conclude that females presenting estrus have higher PRSE than those not presenting estrus. In works that used the protocol based on GnRH+DIP for 7 days, it is reported that the highest PRSE was 48% (Abel et al., 2017) and the lowest was 42% (Nash et al., 2012; Hill et al., 2016a) in cows that do not present estrus prior to AI. In animals that present estrus, the highest PRSE was 71% (Nash et al., 2012) and the lowest 64% (Ferreira et al., 2018). As in
estradiol-based protocols, in those based on GnRH, the presentation of estrus can improve PRSE, since in animals with estrous behavior they are more likely to have mature preovulatory follicle capable of inducing their own ovulation via estradiol. It is recommended, as in heifers, for management reasons and to increase the calving rate at mating end, that FTAI be performed in all animals.

Regarding the effect of ovarian status on PRSE, in females cycling at the beginning of treatment with GnRH+DIP for 7 days, the range of PRSE reported is from 49 % (Marquezini et al., 2013a) to 69 % (Bridges et al., 2014), while, in anestrus animals the range is from 47 % (Nash et al., 2012) to 63 % (Busch et al., 2008). For their part, Giles et al. (2013), using the protocol based on GnRH and DIP for 5 days report that PRSE is 50 % in cycling cows and 52 % in anestrus cows. As mentioned, in the case of heifers treated with GnRH-based protocols, these can induce cyclicity in anestrus animals (Colazo & Mapletoft, 2014; Baruselli et al., 2018), explaining why there is no difference in PRSE between cycling and anestrus cows.
Figure 4. Pregnancy rate to synchronized estrus (PRSE) in female cattle treated with GnRH-based protocols. A) PRSE data distribution in heifers in which a progesterone-releasing intravaginal device (DIP) was used for 5 or 7 days after the first GnRH application. B) PRSE data distribution in lactating cows without the use of a DIP or with its use for 5 and 7 days after the first GnRH application. C) PRSE data distribution at different times between PGF2-α application and AI in lactating cows treated with GnRH-based protocols and the use of a DIP for 7 days. D) PRSE data distribution at different times between PGF2-α application and AI in lactating cows treated with GnRH-based protocols without use of a DIP. Data collected from: Abel et al., 2017; Martínez et al., 2000; Bishop et al., 2017; Bridges et al., 2014; Macmillan et al., 2020; Burns et al., 2008; Busch et al., 2007; Busch et al., 2008; Gruppe et al., 2014; Dahlen et al., 2010; Echternkamp & Thallman, 2011; Esterman et al., 2016; Geary et al., 2001; Giles et al., 2013; Hall et al., 2017; Helguera et al., 2018; Hill et al., 2014; Hill et al., 2016a; Hill et al., 2016b; Kasimanickam et al., 2006; Kasimanickam et al., 2010; Kasimanickam et al., 2014; Kasimanickam et al., 2009; Kasimanickam et al., 2012; Knickmeyer et al., 2019; Lamb et al., 2001; Lamb et al., 2006; Larson et al., 2006; Marquezini et al., 2011; Marquezini et al., 2013a; Marquezini et al., 2013b; Martinez et al., 2002; Mercadante et al., 2015; Mialot et al., 2003; Nash et al., 2012; Oosthuizen et al., 2018a; Oosthuizen et al., 2018b; Oosthuizen et al., 2018c; Rosales-Torres et al., 2017; Small et al., 2009; Stevenson et al., 2003; Thomas et al., 2014; White et al., 2016; Whittier et al., 2010; Whittier et al., 2013; Williams & Stanko, 2020; Wilson et al., 2010; Rodriguez et al., 2023; Bonacker et al., 2020; Rojas-Canadas et al., 2023
Even though the presence of the calf reduces GnRH secretion (Martínez et al., 2000), few studies include temporary weaning as a factor to improve PRSE in the GnRH-based protocols reviewed in this paper. Marquezini et al. (2013a and 2013b) report, in animals treated with GnRH+DIP-based protocols for 7 days, that PRSE is similar when temporarily weaned for 72 hours than when not temporarily weaned. In contrast, Geary et al. (2001) report that temporary weaning for 48 hours tends (P=0.09) to increase PRSE in cows treated with GnRH-based protocols with or without DIP. Although the response to GnRH-based synchronization protocols may be compromised by the presence of the calf and its negative effects on GnRH secretion, as a practical matter and it is based on this evidence, the suggestion is not to temporarily wean.

The use of eCG at the end of GnRH-based protocols is not as common as in estradiol-based protocols. Some reports show, in lactating cows treated with GnRH-based protocols with and without use of DIP, that PRSE is higher in animals where eCG (400 IU) is used at the time of PGF2α application than in cows where eCG is not used (Small et al., 2009; Randi et al., 2021). In contrast, in animals treated with GnRH+DIP-based protocols for 7 days plus temporary weaning, PRSE is not different in animals treated or not with 400 IU eCG at the time of DIP withdrawal (Marquezini et al., 2013b). These results suggest that further studies should be conducted to validate whether the use of eCG can improve PRSE in lactating cows treated with GnRH-based protocols.

**Progesterone-based protocols**

Progesterone-based protocols consist of the use of DIP or progesterone analogs such as MGA for prolonged periods to synchronize estrus in females, followed by PGF2α and GnRH or GnRH, PGF2α and GnRH. The DIP is inserted at day 1 and removed 7, 9 or 14 days later. The purpose of this is that the animals go into estrus and ovulate synchronously. Subsequently, when the animals are in the luteal phase, PGF2α is applied to induce the regression of the CL so that the animals enter proestrus homogeneously (Figure 2G). The most commonly used time, between DIP withdrawal and PGF2α treatment is 16 days, although this hormone can be applied at 11 days (Eborn & Grieger, 2013). After PGF2α application, FTAI can be performed at 66 or 72 hours. A variation to this protocol involves the application of GnRH 7 days prior to PGF2α application (Figure 2H). It is important to note that GnRH can be applied at 2, 4, 9 and 12 days after DIP withdrawal (Mallory et al., 2011; Eborn & Grieger, 2013).

**PRSE in heifers treated with progesterone-based protocols**

In heifers treated with the P-PGF2α-GnRH protocol the lowest reported PRSE was 31 % (Thomas et al., 2017) and the highest was 63 % (Mallory et al., 2011). For heifers treated with the variation of this protocol (P-GnRH-PGF2α-GnRH), the range of PRSE was 44 (Mallory et al., 2011) to 62 % (Busch et al., 2007). From the PRSE data collected from the
selected articles 45% report a PRSE between 61 and 70 % in heifers treated with the P-GnRH-PGF2α-GnRH protocol, while only 25 % of the data are within this range in heifers treated with the P-PGF2α-GnRH protocol (Figure 5A). This suggests, that the P-GnRH-PGF2α-GnRH protocol increases PRSE compared to the P-PGF2α-GnRH protocol. In this regard, Eborn & Grieger (2013), using MGA for 7 days, show that PRSE is higher when GnRH is used after MGA withdrawal (55%) than when it is not used (38 %). Similarly, PRSE is higher in heifers when GnRH is used after DIP withdrawal than in heifers in which it is not used (Kasimanickam et al., 2016). This evidence suggests that use of the P-GnRH-PGF2α-GnRH protocol may improve PRSE because a new wave of follicular growth is synchronized with the use of the first GnRH.

Other factors that may modify PRSE in heifers treated with progesterone-based protocols are the type of progestogen, estrous presentation, and pubertal status. In females treated with the P-GnRH-PGF2α-GnRH protocol, no differences in PRSE were observed between the use of progesterone or MGA (Eborn & Grieger, 2013). Whereas, in heifers that present estrus before PRSE and are treated with the P-PGF2α-GnRH protocol, they have a higher PRSE than when they did not present estrus (Thomas et al., 2014; Thomas et al., 2017). Regarding the pubertal status of heifers, no differences in PRSE were observed between peri-pubertal heifers and pubertal heifers subjected to a progesterone-based protocol regardless of whether or not GnRH was used after progesterone withdrawal (Busch et al., 2007; Mallory et al., 2011). The use of progestogens in these protocols may sensitize the hypothalamus of peri-pubertal animals to induce cyclicity (Perry, 2016). This explains why there is no difference in PRSE between pubescent and peri-pubertal animals when treated with these protocols.

**PRSE in lactating cows treated with progesterone-based protocols**

From the data collected from the selected articles, the lowest PRSE reported in lactating cows treated with the P-GnRH-PGF2α-GnRH protocol was 50 % (Schafer et al., 2007) and the highest was 70 % (Bader et al., 2005). In contrast, cows treated with the P-PGF2α-GnRH protocol the lowest reported PRSE was 46 % (Abel et al., 2017) and the highest was 76 % (Abel et al., 2017). According to the PRSE data shown in Figure 5B, the use of the P-GnRH-PGF2α-GnRH protocol in lactating cows appears to increase PRSE compared to cows treated with the P-PGF2α-GnRH protocol. This is because the use of GnRH prior to PGF2α application synchronizes a new wave of follicular growth.
Figure 5. Pregnancy rate to synchronized estrus (PRSE) in female cattle treated with progesterone-based protocols. A) Distribution of PRSE data in heifers in which GnRH was or was not applied after progesterone withdrawal. B) Distribution of PRSE data in lactating cows in which GnRH was or was not applied after progesterone withdrawal. Data compiled from Bader et al., 2005; Busch et al., 2007; Eborn & Grieger, 2013; Schafer et al., 2007; Mallory et al., 2011; Martin et al., 2014; Kasimanickam et al., 2016; Stegner et al., 2004; Thomas et al., 2014; Thomas et al., 2017; Ketchum et al., 2021

In lactating cows submitted to these protocols, PRSE is higher when animals present estrus before FTAI than when they do not present estrus (Thomas et al., 2014). In lactating cows treated with the P-PGF2α-GnRH protocol PRSE was 71 and 76 % when they presented estrus before insemination while when they did not present estrus PRSE was 43 and 53 % (Abel et al., 2017). Regarding postpartum ovarian status, Schafer et al. (2007) using the P-GnRH-PGF2α-GnRH protocol reported that PRSE is similar between cycling cows (59 %) and cows in postpartum anestrus (64 %). Similarly, in cows treated with the same protocol, regardless of whether MGA is used for 7 or 14 days, PRSE is similar between cycling and postpartum anestrus cows (Bader et al., 2005). As in heifers, the presentation of estrus in lactating cows ensures the presence of a mature preovulatory follicle capable of ovulation when GnRH is applied in conjunction with AI. Whereas, progesterone-based protocols as well as GnRH-based protocols can induce ovarian activity in postpartum anestrus cows.
Conclusions

Currently used estrous and ovulation synchronization protocols manipulate follicular development with the use of estrogens, GnRH or progestogens to subsequently induce ovulation of a preovulatory follicle for FTAI. In addition, it must be ensured that the animals enter a follicular phase synchronously using PGF2α and DIPs. Based on the information reviewed in this work, the reported PRSE data are highly variable, however, independent of the protocol type used, it is possible to obtain PRSE of between 40 and 60%. Depending on the protocol type used and the physiological status of the animal, some factors can be used to improve PRSE. In lactating cows the use of eCG increases PRSE when included in estradiol-based protocols, while in heifers and lactating cows treated with GnRH-based protocols, PRSE can be increased by using DIP for 5 or 7 days. Finally, in females treated with progesterone-based protocols, the use of GnRH prior to PGF2α application improves PRSE.

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