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REVIEW

GnRH agonists: Updating fixed-time artificial insemination protocols in sows

María Victoria Falceto[1] | Andrés Suárez-Usbeck[2,3] | María Teresa Tejedor[4] | Raquel Ausejo[2] | Ana María Garrido[2] | Olga Mitjana[1]

[1]Agroalimentary Institute of Aragon-IA2, Department of Animal Pathology, Universidad de Zaragoza-CITA, Zaragoza, Spain

[2]Department of Animal Pathology, Universidad de Zaragoza, Zaragoza, Spain

[3]Facultad de Ciencias Pecuarias, Escuela Superior Politécnica de Chimborazo, Riobamba, Ecuador

[4]Department of Anatomy, Embriology and Animal Genetics, CiberCV, Universidad de Zaragoza, Zaragoza, Spain

Correspondence
Olga Mitjana, Agroalimentary Institute of Aragon-IA2, Department of Animal Pathology, Universidad de Zaragoza-CITA, C/Miguel Servet 177, Zaragoza 50013, Spain.
Email: omitjana@unizar.es

Abstract

Protocols for fixed-time artificial insemination (FTAI) in swine reproduction can help increase genetic improvement and production efficiency. Different gonadotropin-releasing hormone (GnRH) agonists have been developed to gain better control of follicular development, timing, and ovulation quality; therefore, they have been extensively used in FTAI protocols. This literature review resumes the most important characteristics of the physiology of follicular development and ovulation in sows, followed by a discussion about the hormonal alternatives available to induce ovulation (human chorionic gonadotropin, hCG; porcine luteinizing hormone, LH and GnRH agonists). Also, ovulation induction failures with GnRH agonists are described. Finally, current FTAI protocols with GnRH agonists are resumed and discussed. FTAI with GnRH agonists has proven to be an efficient, successful reproductive protocol that can be implemented in pig farms due to better knowledge of an endocrine system that regulates follicular development and ovulation and increased availability of several GnRH agonists that allow more efficient reproductive swine programs.

KEYWORDS
FTAI, GnRH agonists, hormones, sows

1 | INTRODUCTION

Some reproductive hormones used to control follicular development and ovulation have been available in the swine industry for the past 60 years. However, they are rarely used except when treating certain cases of anestrus (Hühn et al., 1996). Some of the reasons for their limited use in the past include high cost and poor practical application on commercial farms (Hayden, 2008; Knox, 2015).

Currently, the swine industry is greatly interested in the use of gonadotropin-releasing hormone (GnRH) agonists. Several studies have been carried out to define fixed-time artificial insemination (FTAI) protocols in sows using GnRH agonists (Brüssow et al., 1990; Knox et al., 2003; Martinat-Botté et al., 2010; Von Kaufmann & Holtz, 1982). Applying it to farm reproductive control protocols can help increase genetic improvement and swine production efficiency (Baroncello et al., 2017; Pearodwong et al., 2019; Rodrigues et al., 2020; Suárez-Usbeck et al., 2021).

Knowledge of GnRH agonists began in the early 20th century when it was discovered that pituitary lesions, specifically in the adenohypophysis, led to atrophy of the genital tract, thus identifying the hypothalamic–hypophysis–ovarian (H-H-O) axis. In 1928, Louria & Rosenzweig demonstrated the gonadal stimulation function using urine samples obtained from pregnant women that showed the presence of human chorionic gonadotropin (hCG). Subsequently, Fevold et al. (1931) provided the first evidence of the existence of two

pituitary gonadotropins, which led to the purification and isolation of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). During the following 30 years, ovarian stimulation with exogenous gonadotropins was performed using pregnant mare serum gonadotropins (PMSGs), equine chorionic gonadotropins (eCG), and human pituitary gland extracts. These exogenous gonadotropins were used to obtain ovulation induction, although the formation of antibodies and the poor biosecurity limited their commercial use in veterinary medicine (Hayden, 2008).

Tanabe et al. (1949) were the first to use eCG with pituitary extracts from sheep to stimulate follicular development and induce ovulation in sows. Other studies subsequently formed the basis for the development of estrus and ovulation protocols in swine production, including Polge and Day (1969), who used methallibure to restrict follicular growth and eCG treatment to stimulate follicular development and hCG for ovulation induction.

In the 1960s, it was possible to obtain FSH and LH extracts from the pituitary glands of various animals, although due to poor biosafety and purity of the hormones, little pharmacokinetic efficacy was obtained (Hayden et al., 1999). These preparations began to be used were used to cause induction of ovulation in the sow. In recent years, the gonadotropins used to control the estrus and ovulation in swine production have been eCG, a glycoprotein with FSH and LH-like activity, and hCG, a glycoprotein with a high LH effect (Farmer & Papkoff, 1979; Kirkwood, 1999). A common protocol for estrus stimulation in sows is to inject 400 IU of eCG and 200 IU of hCG, which has demonstrated high efficacy in inducing estrus after weaning or estrus synchronization with altrenogest but does not provide ovulation synchronization (Kirkwood, 1999). In addition, to induce an earlier onset estrus, the estrus–ovulation interval is prolonged, which makes predicting ovulation time significantly more difficult (Estienne et al., 2001; Knox et al., 2001). One inconvenience of using a combination of eCG and hCG at higher doses (700 and 350 IU, respectively) is the risk of ovarian cyst production, follicle luteinization, and cystic ovarian degeneration (Brüssow & Wähner, 2001).

The duration of estrus is highly variable among sows; therefore, exogenous gonadotropins are useful for synchronizing ovulation. In 1971, hypothalamic GnRH decapeptide was isolated, allowing researchers to identify specific regions of the adenohypophysis to determine the activation and the stability of its binding to the pituitary GnRH receptor (Garcia et al., 2004; Hayden, 2008; Schally, 1999). Subsequently, gonadorelin was one of the first GnRH agonists synthesized in East Germany in the late 1970s (Brüssow & Bergfeld, 1979). Since then, GnRH agonists have been used to stimulate follicle development and induce ovulation in both nulliparous and multiparous sows (Lopez, 2009). GnRH agonists are considered a possible alternative to gonadotropin administration because they act at the pituitary level and stimulate LH and FSH release (Brüssow & Wähner, 2001), allowing them to carry out FTAI at a time close to ovulation (Kirkwood & Kauffold, 2015).

For many years, different GnRH agonists have been developed to gain better control of follicular development, timing, and ovulation quality to improve reproductive protocols in commercial farms. For FTAI protocols with different GnRH agonists that are commercially available, it is essential to understand the reproductive physiology and, specifically, the process of follicular development and ovulation in swines (Knox, 2015).

2 | PHYSIOLOGY OF FOLLICULAR DEVELOPMENT AND OVULATION IN SOWS

The estrous cycle is composed of a luteal phase (approximately 16–18 days) and a follicular phase (approximately 3–6 days). During the luteal phase, the corpora lutea (CL) produce progesterone (P4) to limit FSH and LH secretion and halt follicular development (Falceto, 2016). Around 12–14 days of the luteal phase, the uterine produces prostaglandin F2α (PGF2α), causing the CL to regress and the progesterone production to disappear (Kirkwood & Kauffold, 2015). Reactivating the H-H-O axis allows the GnRH to resume and release LH and FSH hormones for follicular growth, which initiate the next estrus cycle when combined with an increase in oestrogen (E2), (Manjarin et al., 2009).

2.1 | Control of GnRH and gonadotropin production

Although not all species respond similarly, for most species, positive feedback of estradiol (E2) on the H-H-O axis is a key which initiates the next estrous cycle. In pigs, E2 acts in the hypothalamus to modulate GnRH release and stimulate production of gonadotropins in pituitary (hypophysis) (Brüssow et al., 2009; Knox, 2015) (Figure 1). In addition, E2 has been shown to induce a temporary decrease in LH, which allows the pituitary to accumulate LH reserves for the subsequent 24–48 h ovulatory surge (Beltramo et al., 2014; Pearodwong et al., 2019).

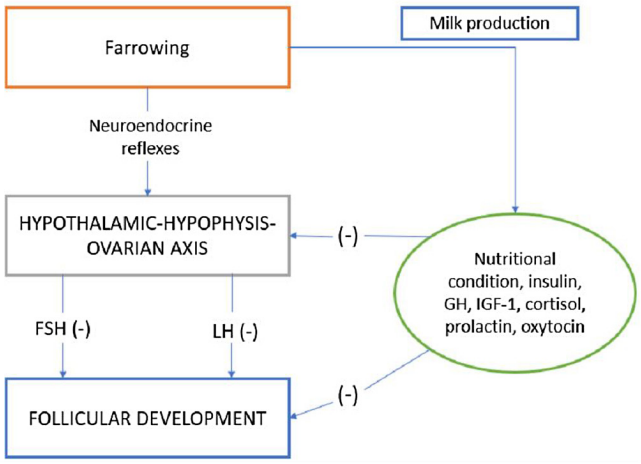

FIGURE 1 Hypothalamic–hypophysis–ovarian axis in sows after farrowing and during lactation. The symbol (–) indicates negative feedback.

GnRH is released in pulses of different frequency and amplitude that are regulated by the H-H-O axis. Ovarian feedback regulation of FSH and LH secretion occurs through the hypophysis GnRH receptors. GnRH pulses reach the pituitary through portal pituitary vessels and induce FSH and LH release from gonadotropic cells (Tsutsui & Ubuka, 2014).

GnRH has a short half-life in blood and is eliminated within 4 min, mainly by glomerular filtration (Chamson-Reig et al., 2003; Tzoupis et al., 2020). In swine, the frequency and amplitude of GnRH pulses affect LH and FSH release patterns. During the onset of the follicular phase, E2 levels increase, which increases the frequency and decreases the amplitude of GnRH pulses (Smith, 2008), increasing the frequency of LH and FSH release (Knox et al., 2003). During the luteal phase, GnRH release occurs in high-amplitude, low-frequency pulses and P4 levels increase. At the end of the luteal phase, GnRH frequency pulses increase again and P4 levels decrease, restarting the estrous cycle. The pulsatile GnRH release is relevant because it modifies LH and FSH synthesis, causing the correct development of the follicles and the correct functioning of the H-H-O axis (Thompson & Kaiser, 2014).

2.2 | Ovarian receptors for pituitary FSH and LH gonadotrophins

In sows, primordial follicles leave the resting phase and develop in the antral phase, where they become dependent on FSH for growth and survival (Knox, 2015). Only 15% of follicles evolve to the next stage of follicular development, while the rest undergo atresia due to cellular apoptosis of the granulosa cells (Guthrie & Garrett, 2001).

When follicles reach approximately 1 mm, they become visible on the ovary surface. Follicles that are small to medium size predominantly bind FSH receptors; which receptors decrease as follicle grow and mature (Foxcroft & Hunter, 1985).

The expression of FSH and LH receptors varies depending on the development of each ovarian follicle. Small follicles (1 mm) show maximal FSH and low LH receptors expression. In medium-sized follicles (between 1–6 mm), gonadotropins can bind to both FSH and LH receptors. In large follicles (6–12 mm), LH receptor expression and binding are very high, whereas FSH receptor expression is not detectable (Knox, 2015).

2.3 | Follicular development during the estrous cycle in sows

Prepubertal gilts have been shown to initiate responses to gonadotropin FSH around 60 days of age, coinciding with the appearance of ovarian FSH receptors, although the H-H-O axis of gilts does not begin functioning until after 100 days of age and reaches puberty around 7 months of age (Rátky et al., 2005; Schwarz et al., 2013). On the other hand, in mature gilts, the follicular cohort selected to ovulate is stimulated by the FSH surge and influenced by a high concentration of P4 present during the diestrus (Prunier & Quesnel, 2000; Schwarz et al., 2008). It takes place only after corpora lutea (CL) luteolysis and causes P4 drop without the negative feedback on gonadotropin synthesis, allowing the medium-size ovarian follicles to grow to pre-ovulatory size in 4–6 days. Therefore, there must be a balance between the stimulatory (LH and FSH) and inhibitory (P4 and inhibin) factors of the H-H-O axis to ensure efficient reproductive function. Multiple internal and external factors are involved in the estrous cycle (Brüssow & Wähner, 2001) (Figure 1).

Once follicles reach a preovulatory stage, the FSH decreases and the pulsatile high-amplitude, low-frequency LH secretion changes to a low-amplitude, high-frequency pattern (Thompson & Kaiser, 2014). At this stage, LH receptor expression and E2 production increase, triggering the LH surge mediated by GnRH and initiating ovulation (Brüssow & Wähner, 2001), which is characterized by ruptured vessels and destroyed connective tissue in the follicle wall that releases mature oocytes for fertilization (Mellagi et al., 2010). Time from onset of estrus to beginning of LH preovulatory surge varies 8 ± 11 h; duration of the surge lasts about 24 h, with 30 ± 3 h elapsed from onset to ovulation (Knox, 2015).

There is evidence that not all follicles in the preovulatory phase are the same size; the differences in the number of FSH receptors involved in follicular development and the influence of various external factors on the H-H-O axis are dependent on the sow (e.g., negative energy balance, stress, farrowing, and reproductive seasonality). This can trigger fertility failure, poor embryonic survival, or cystic follicles (Knox et al., 2010, 2014) and small CL (luteal insufficiency) (Falceto, 2016).

Although all preovulatory follicles respond to LH surge, there is asynchronous ovulation timing among them, ranging from 1–3 h from the first to the last oocyte (Hunter et al., 2004). According to Kemp and Soede (1996) and Tummaruk et al. (2011), ovulation occurs after 70%–72% of estrus onset, regardless of its duration. However, Almeida et al. (2000) shows that ovulation can even occur after 85% of estrus onset in gilts.

2.4 | Follicular development during sow lactation

Sows show lactational anestrus after farrowing. Although a cohort of 20–30 follicles (2 mm) is selected in the ovary, they only grow up to 4 mm and subsequently become atretic (Lucy et al., 2001) because at the lactation stage, GnRH secretion is inhibited due to the actions of prolactin, oxytocin, and endogenous opioid peptides that produce negative feedback on the H-H-O axis (Varley & Foxcroft, 1990).

2.5 | Post-weaning follicular development

During weaning, sows usually have several 2–5 mm diameter follicles available to develop into pre-ovulatory follicles for the next estrus

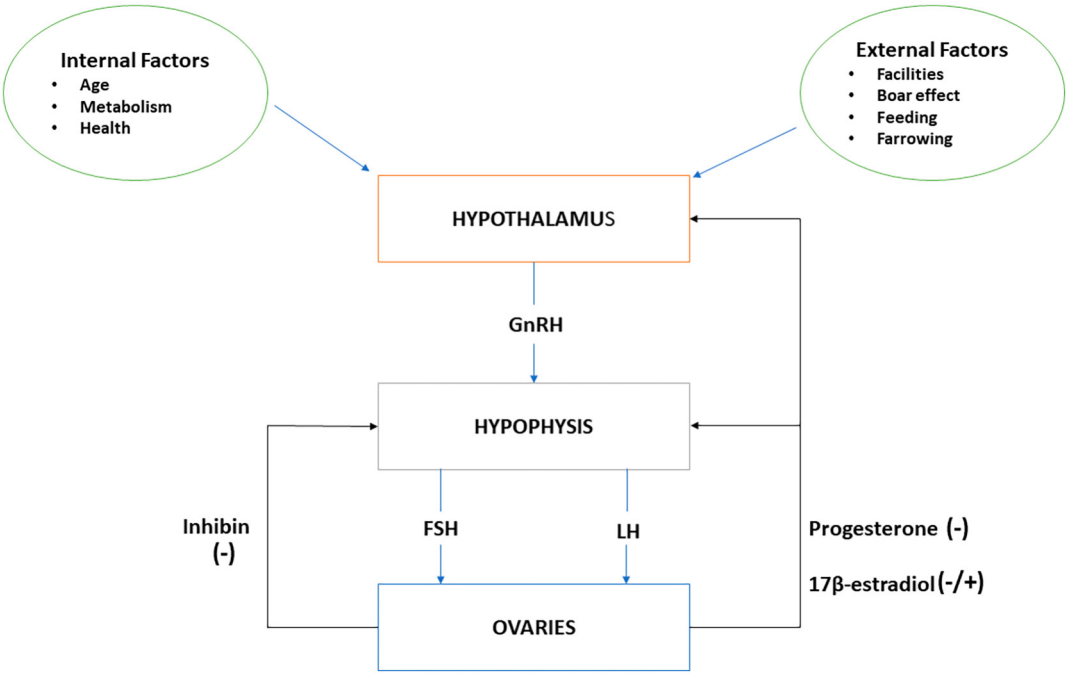

FIGURE 2 Effect of lactation on the hypothalamic–hypophysis–ovarian axis.

cycle (Lucy et al., 2001). Once the piglets are weaned, the follicles reach 6–7 mm, and the sows come out in estrus (Liu et al., 2000; Lucy et al., 2001) and subsequently reach the pre-ovulatory size of 8–12 mm (Falceto, 2016) (Figure 2).

Regulating factors of follicle development that are released under stress (e.g., corticosteroids), nutritional mediators (e.g., glucose, insulin, and fatty acids), and endogenous opioids and leptin (Kirkwood & Kauffold, 2015) affect the wean-to-estrus interval and wean-to-ovulation interval (Lucy et al., 2001). Initially after weaning, FSH rises and then falls, whereas LH increases both the amplitude and frequency of pulses to start the next post-weaning estrus cycle.

3 | INDUCTION AND SYNCHRONIZATION OF OVULATION IN SOWS

After reviewing the follicular dynamics of gilts and weaned sow, three different hormonal alternatives available to induce ovulation in sows were identified: hCG, porcine LH, and GnRH agonist.

Altrenogest is a progesterone commonly used in combination with gonadotropins or gonadotropin-releasing hormone agonists; it is widely used to synchronize gilts for different reproductive strategies of insemination (Brüssow et al., 2009; Driancourt et al., 2013; Martinat-Botté et al., 2010). On the other hand, this treatment can cause follicular cyst formation, especially in prepubertal gilts. Ziecik et al. (2020) showed that in prepubertal gilts, altrenogest decreased the percentage of primordial and atretic small follicles increasing large antral follicles, while in mature gilts, the percentage of primary follicles was reduced and the total number of preovulatory antral follicles was elevated by altrenogest action. These researches studied the effects of altrenogest on the ovarian follicle development in gilts and concluded that altrenogest negatively affected follicular fluid progesterone concentration and decreased levels of prostaglandin (E2) in prepubertal gilts and PGF2 alpha metabolite in mature gilts. Metabolism of cAMP in granulose cells of mature gilts was altered by altrenogest. Altrenogest also acts at genetic level by downregulation of *CYP17A1* mRNA in the prepubertal theca layer and PGF2 alpha synthase expression in the granulosa and theca layer of mature gilts. Recent studies described that altrenogest administration in combination with gonadotropins had negative impacts on fertility and embryo viability for multiparous sows (Gonzalez-Ramiro et al., 2021, 2022).

3.1 | Human chorionic gonadotropin (hCG)

In the 1970s, a treatment with 800–1000 IU of equine chorionic gonadotropin (eCG) (24 h after weaning/altrenogest) and human chorionic gonadotropin (hCG) was administered 78–80 h after eCG treatment. Ovulation occurred approximately 40–42 h later hCG injection. Therefore, sows were inseminated at a fixed time after hCG injection (14–16 h in gilts and 24–26 h in multiparous sows). (Brüssow et al., 2009). Different insemination moment was selected in gilts and sows since these groups differ in follicle development and oocytes survival time; gilts are immature and their oocytes only survive for 4 h, while oocytes from multiparous sows survive for 8 h (Brüssow et al., 2009). Cassar et al. (2005) indicated that the appropriate hCG dose for multiparous sows was 600 IU. However, a dose of 900 IU produced a better performance in follicular development for gilts (Cassar et al., 2010). Administering 750 IU of hCG 80 h after weaning without a prior eCG injection has also been shown to be effective;

TABLE 1 FTAI protocols with gonadorelin and reproductive performances after eCG application.

Sow parity	*n*	GnRH dose μg	Interval weaned/Altrenogest–gonadorelin (h)	Interval gonadorelin-AI (h)	nof AI	Farrowingrate%	Total born piglets	Born alive piglets	Country	References
Gilts	1285	50	80	40–42	2	78.8	10.4 ± 7.0	9.92 ± 6.0	Germany	Brüssow et al., 1996
Multiparous	20,701	50	55–58	42	2	83	11.6 ± 0.9	11 ± 1.0	Germany	Brüssow et al., 1996
Multiparous	19,954	25	55–58	25–26	2	81.7	11.6 ± 2.0	11 ± 0.7	Germany	Brüssow et al., 1996
Gilts	54	150	80	23–25	1	88.9	10.4 ± 0.3	9.7 ± 0.3	Canada	Kirkwood, 1999
Multiparous	51	50	24 and 72	-	3	94	10.1 ± 3.0	9.1 ± 3.0	Mexico	Romo et al., 2005

Note: Data about total born piglets and born alive piglets are mean ± SEM (standard error of mean).

compared to the historical herd data (75.7%), fertility rates were improved by 92.3% (a difference of 16.2%) (Cassar et al., 2004).

3.2 | Porcine LH

Porcine LH is a gonadotropin used to control the timing of ovulation in weaned sows (Candini et al., 1999). Ovulation occurs approximately 38 h after injection of 5 mg LH (Cassar et al., 2005). Protocols including pretreatment with eCG resulted in a 15% increase in farrowing rates when compared to treatments using only hCG (Bennett-Steward et al., 2008; Cassar et al., 2005). These results demonstrated the potential of LH for ovulation control on commercial farms by exceeding the hCG protocol results.

3.3 | GnRH agonists

The discovery of the GnRH analog's structure and synthesis led to it becoming a potential substitute for hCG and eCG in follicular growth stimulation and ovulation induction (Brüssow et al., 1996). The following GnRH agonists have been tested to stimulate LH secretion and induce ovulation in sows: gonadorelin (Brüssow et al., 1990, 1996), lecirelin (Baruselli et al., 2001); peforelin (Brüssow et al., 2010; De Jong et al., 2017; Hunter et al., 2004), goserelin (Brüssow et al., 2007), buserelin (Driancourt et al., 2013; Martinat-Botté et al., 2010), and triptorelin (Knox et al., 2011, 2017).

Application of GnRH to induce LH release has several advantages when compared with hCG treatment. While hCG directly acts on ovarian receptors, GnRH stimulates LH release by the pituitary gland, which then reaches the ovary to help in the ovulation process. Also, treatments including exogenous gonadotropins and altrenogest can cause ovarian follicular cysts in gilts (Ziecik et al., 2021).

GnRH agonists have a higher affinity for adenohypophysis receptors than natural GnRH, remaining bound longer and stimulating FSH and LH secretion. They have a prolonged half-life, which makes them more efficient than endogenous GnRH (Fries et al., 2010). The agonist's design is intended to stabilize the molecule against enzymatic attack, increase its binding to plasma proteins and membranes, and increase its affinity to the GnRH receptor (Lopes et al., 2020). In swine production, GnRH agonists are used for ovulation induction afterestrus synchronization by altrenogest (15–20 mg/day/sow for 14–18 days) in gilts or weaned multiparous sows to achieve an additional synchronization effect that can be used to fulfil FTAI protocols (Kirkwood & Kauffold, 2015).

Concerning the administration route, the time of application for artificial insemination (AI) can differ depending on the molecule (Suárez-Usbeck et al., 2021). Results may vary due to factors of follicle development related to induction protocols, type of analog, doses used, and timing of administration (Knox, 2015; Pearodwong et al., 2019). The agonists used in recent years for swine reproduction include the following: gonadorelin, licerelin, peforelin, buserelin, and triptorelin.

3.3.1 | Gonadorelin

Gonadorelin ($C_{55}H_{75}N_{17}O_{13}$) was the first GnRH agonist molecule developed in East Germany. Following eCG administration, 50 μg was injected to induce ovulation approximately 36 h later (Brüssow et al., 1990), which improved fertility outcomes compared to the protocol that administered eCG and hCG simultaneously (Brüssow et al., 1996). Gonadorelin-induced estrous and ovulation were observed in gilts (Lutz et al., 1985) and in sows during lactation and postpartumanestrus (Britt et al., 1985). It had excellent reproductive and fertility results, farrowing rates, and litter quality (Brüssow et al., 1996; Rubio et al., 2009). However, Romo et al. (2005) showed that supplementing methionine with 50 μg of gonadorelin 4 days before weaning had no significant effect on fertility or farrowing rate compared to using methionine alone.

Gilts treated with 50 μg of gonadorelin began ovulating 35.5 ± 2.7 h after treatment and finished59 ± 1.7 h later. However, sows varied in their response with respect to the interval between GnRH injection and LH surge. A total of 1285 gilts were injected with 50 μg of gonadorelin 78–80 h after 1000 IU of eCG and FTAI twice (24 and 40 h after gonadorelin administration). They presented high fertility results with these ovulation induction protocols. (Brüssow et al., 1996). Table 1 shows the results of the protocols performed with gonadorelin.

3.3.2 | Licerelin

Licerelin ($C_{59}H_{84}N_{16}O_{12}$) is a long-acting synthetic GnRH agonist obtained by modifying the structure of gonadorelin (Baruselli et al., 2001). The efficacy of 25 μg of licerelin in ovulation induction for weaned sows showed a reduction of the estrus period and the interval between the estrus onset and ovulation. Moreover, 93% of the treated sows ovulated at 48 h post-treatment, obtaining good reproductive performances (Fries et al., 2018).

3.3.3 | Peforelin

Peforelin ($C_{59}H_{74}N_{18}O_{14}$) is a GnRH analog that stimulates endogenous FSH secretion. After injection, peforelin is rapidly absorbed and has a prolonged half-life. It is used for estrus induction but not for ovulation induction in sows due to the additional stimulation of FSH secretion (Brüssow et al., 2010).

Engl (2006) obtained good results with a dose of 37.5 μg for primiparous sows. Nowadays, however, preferred peforelin dosage is 150 μg for both gilts and multiparous sows according to manufacturer instructions and researcher studies (De Jong et al., 2013), the only difference being the time of application. For gilts, it is administered 48 h after treatment with altrenogest; for multiparous sows, it is administered 24 h after weaning (De Jong et al., 2013)., Administering peforelin produced an increase in follicle diameter due to increased FSH during the follicular phase that influenced the size of CL and P4 levels. In addition, homogeneous litters and higher birth weight were obtained through the peforelin treatment rather than the control treatment (1.42 ± 0.38 vs. 1.35 ± 0.35), although the number of live births was lower compared to the control group (12.8 ± 3.3 vs. 13.2 ± 3.6) (de Jong et al., 2017; Hunter et al., 2004).

3.3.4 | Buserelin

Buserelin ($C_{62}H_{90}N_{16}O_{15}$) is a GnRH agonist, currently used, in sows, where the main compound is buserelin acetate. Studies by Von Kaufmann and Holtz (1982) demonstrated that administering 10 μg of buserelin efficiently induces ovulation in gilts pretreated with eCG. In some later studies, buserelin was administered at different times (24, 77, 94, and 104 h after weaning) and at different doses (6, 10, 16, 16, and 50 μg) (Driancourt et al., 2013; Martinat-Botté et al., 2010; Wongkaweewit et al., 2012). Currently, the recommendation for multiparous sows is an intramuscular injection of 10 μg buserelin 83–89 h after weaning and a single insemination 30 to 33 h later, with ovulation occurring 42 ± 2 h post-buserelin (Baroncello et al., 2017; Falceto et al., 2014; Lopes et al., 2020; Pearodwong et al., 2019).

For gilts, Driancourt et al. (2013) explained that the time of ovulation occurs 35–41 h post-treatment with buserelin, administered 86 ± 3 h after weaning. Martinat-Botté et al. (2010) induced gilts with 10 μg of buserelin 115–120 h after the last altrenogest treatment and inseminated twice 30–33 h later. Suárez-Usbeck et al. (2021) performed ovulation inductions with buserelin on gilts 120 h after altrenogest treatment using a single post-cervical artificial insemination (PCAI) at fixed times (30–33 h after buserelin injection), obtaining excellent reproductive performances. Table 2 shows the research carried out with buserelin.

3.3.5 | Triptorelin

Triptorelin ($C_{64}H_{82}N_{18}O13$) is a synthetic GnRH analog for intravaginal application that stimulates the adenohypophysis to secrete LH and induce ovulation (Gesing, 2015; Knox et al., 2014). It is commonly used in the United States and Canada in FTAI protocols; weaned sows receive a single dose regardless of whether they show signs of estrus (Knox et al., 2017; Kraeling & Webel, 2015).

Ovulation occurs 48 h after triptorelin treatment for 81% of weaned multiparous sows and 92.6% in weaned primiparous sows (Gesing, 2015; Knox et al., 2014) however, the results are variable (Knox et al., 2011) andeven worse (Fabi, 2017; Merdy et al., 2022; Rodrigues et al., 2020). The variations could originate from the absence of estrus detection before performing FTAI, differences in triptorelin doses, or timing of injection. AI. Wang et al. (2020) performed a meta-analysis based on a total of 37 trials from 15 studies, carried out between 2004 and 2018. This meta-analysis included randomized controlled trials and clinically controlled trials that studied gilts or sows with all data (except for total born

TABLE 2 FTAI protocols and reproductive performances with buserelin.

Sow parity	*n*	Buserelin dose μg	Interval weaned/ Altrenogest Buserelin (h)	Interval Buserelin –AI (h)	nof AI	Farrowing rate%	Total born piglets	Born alive piglets	References
Multiparous	15	10	104	30–33	2	71.4	15.6±2.4	14±1.6	Martinat-Botté et al., 2010
Multiparous	13	10	94	30–33	2	84.6	14±3.2	12.5±2.5	Martinat-Botté et al., 2010
Gilts	184	10	115–120	30–33	2	78.8	13.1±0.3	12.1±0.3	Driancourt et al., 2013
Multiparous	174	10	86–89	30–33	2	88.1	13.6±0.3	12.6±0.3	Driancourt et al., 2013
Gilts	39	10	86–89	30–33	2	78.1	13.2±0.8	12.7±0.8	Driancourt et al., 2013
Multiparous	1000	10	83–89	30–33	1	90.0	12.6	11.4	Falceto et al., 2014
Multiparous	165	10	86–89	30–33	1	83.9	12.9±0.3	-	Baroncello et al., 2017
Multiparous	43	10	86–89	30–33	2	83.3	11.9±0.5	10.9±0.6	Pearodwong et al., 2019
Multiparous	88	10	86	30–33	2	85.3	-	13.2±0.3	Lopes et al., 2020
Gilts	238	10	115–120	30–33	1	91.1	18.1±0.3	17.04±0.3	Suárez-Usbeck et al., 2021

Note: Data about total born piglets and born alive piglets are mean±SEM (standard error of mean).

and born alive piglets' data) expressed as either mean±SE or mean±SD. Studies that were not published as full reports were excluded. Conference papers were cross-checked with journal papers.This meta-analysis concluded that the right moment for the application of triptorelin is 96 h after weaning, demonstrating a significant effect on farrowing rate ($p < .001$). Doses of 100 μg proved to be better than doses of 200 μg, with a positive effect on fertility and farrowing rates ($p < .05$). The correct time for insemination was determined to be 24 h for the first dose and 48 h for the second dose after intravaginal triptorelin application. Although new studies are needed regarding its application in gilts, these results suggest that triptorelin administration would be successfully added to current FTAI protocols.

Sows treated with triptorelin displayed similar reproductive performances as the control sows because the ovulation timing was accurately predicted by assuming that the two FTAI should be performed when estrus has been detected (Knox et al., 2014; Ulguim et al., 2016). On the other hand, Knox et al. (2018) and Wang et al. (2020) determined in their research (experimental and meta-analysis, respectively) that administering 100 μg triptorelin at 96 h to weaned sows results in improved reproductive parameters compared to triptorelin treatments on gilts synchronized with altrenogest.

One of the main purposes of triptorelin application is to perform FTAI without detecting estrus; however, in these studies, inseminations were performed as estrus was observed. In all experiments, the conclusions mention that more field studies should be carried out to replicate results and obtain more accurate conclusions.

Some research observed in Wang et al. (2020) meta-analysis, which was conducted by applying different doses of triptorelin (25, 100, and 200 μg) to gilts, presented low reproductive parameters (i.e., fertility rate, farrowing rates, total births, and still births) compared to the control sows, which may be due to two reasons. First, the gilts in the triptorelin group received a single insemination at a fixed time, while those in the control group received multiple inseminations (Gesing, 2015; Wang et al., 2020). Second, triptorelin was administered very soon (72, 84, 96, and 120 h) after altrenogest treatment, resulting in an ovulation rate of 70.9% compared to 92.5% on day 6 post-treatment (Wang et al., 2020). Table 3 shows the results of the protocols performed with triptorelin.

4 | OVULATION INDUCTION FAILURES WITH GnRH AGONISTS

The optimal time to achieve excellent fertility rates with sows is by inseminating 24 h prior to ovulation. However, there is a large variation among sows in the wean-to-estrus interval, the duration of estrus, and, consequently, the onset of estrus-ovulation interval (Cassar et al., 2005; De Rensis et al., 2003), which presented a challenge in determining the optimal time for AI protocols in commercial farms. Variation in the onset of estrus and ovulation could be associated with the heterogeneity of follicle development and LH surge response of follicle development (Knox, 2015; Nissen et al., 1997). The lifespan of oocytes after ovulation is 8–12 h, and the lifespan of sperm capable of fertilization is 24 h; these characteristics define the

TABLE 3 FTAI protocols with triptorelin and reproductive performance results.

Sow parity	n	Triptorelin dose µg	Interval weaned/ Altrenogest triptorelin(h)	Interval triptorelin–AI(h)	nof AI	Farrowing rate%	Total born piglets	Born alive piglets	References
Multiparous	502	100	96	24	3	90.2	12.5±0.9	-	Baer & Bilkei, 2004
Multiparous	56	100	96	8 and 32	2	83.3	12.1±0.8	10.6±1.4	Roski, 2004
Multiparous	84	100	96	8 and 32	2	83.3	11.7±0.5	10.1±0.4	Stewart et al., 2010
Multiparous	503	100	96	8 and 24	2	73.3	11.1±1.7	9.2±0.6	Knox et al., 2011
Multiparous	131	25	96	Estrus	1	79.1	13.2±0.5	11.5±1.1	Knox et al., 2014
Multiparous	126	100	96	24–28	1	70.8	12.3±0.5	11.2±0.8	Knox et al., 2014
Multiparous	113	200	96	24	1	76.6	12.4±1.1	11±0.7	Knox et al., 2014
Multiparous	2314	-	-	-	1	89.9	13.2±1.2	12.1±0.2	Knox et al., 2018
Multiparous	48	200	96	24	1	62.1	9.9±1.6	8.8±0.3	Fabi, 2017
Multiparous	478	-	96	24 and 48	2	93.5	14.7±0.2	13.4±0.2	Dillard et al., 2018
Gilts	61	100	144	24	1	89.5	-	-	Rodrigues et al., 2020
Multiparous	204	100	96	22–23	1	91.4	-	-	Renaud et al., 2020
Multiparous	70–168	100	96	24	3	92.1	14.3	-	Merdy et al., 2022

Note: Data about total born piglets and born alive piglets are expressed as mean±SEM (standard error of mean). In all these studies, estrous detection was performed and only sows/gilts showing estrous were inseminated.

time during which AI protocols can lead to successful fertilization (Cassar et al., 2005). The use of GnRH agonists for estrus synchronization and ovulation induction allows for improved estrus control and more accurate determination of ovulation time to correctly perform AI (Cassar et al., 2005).

Choosing the correct timing of GnRH agonist administration for ovulation induction (either after progestogen administration or after weaning) ensures treatment success because the LH level induced by GnRH agonist administration is quite similar to the LH surge induced by endogenous GnRH (Degenstein et al., 2008; Driancourt et al., 2013).

The results indicate that GnRH agonists are generally effective at inducing ovulation, although some authors report that 16% of sows did not ovulate 48 h after applying any GnRH agonist (Knox, 2015; Knox et al., 2017). From a practical point of view, implementing a careful FTAI protocol could help improve fertility of nulliparous sows (Brüssow et al., 2009; Fries et al., 2010; Pearodwong et al., 2019). Suárez-Usbeck et al. (2021) indicated that 11% of nulliparous sows did not come out in estrous after buserelin application.

Treatment failures with GnRH agonists are due to two main reasons. First, the LH preovulatory surge may not reach the threshold value necessary to activate the ovulatory process. Second, the LH surge signal may not occur at the expected time (Castagna et al., 2004), leading to embryo losses from performing inseminations during late estrus (Rozeboom et al., 1997). However, in most cases, the absence of ovulation is related to applying GnRH agonists to sows that are not in the proestrous phase of the estrous cycle either after progestogen administration or after weaning. This means the gilts are prepubertal, in post-weaning anestrus, or have had an undetected estrus before weaning and are in the diestrus phase with the presence of CL, which prevents the ovary from responding to the hormone (Falceto, 2018).

5 | CURRENT PROTOCOLS FOR FIXED-TIME ARTIFICIAL INSEMINATION (FTAI) WITH GNRH AGONISTS

To properly perform ovulation inductions, researchers must carefully determine the timing of GnRH agonist administration, the time at which sows show estrus signs, and the timing of AI. As previously indicated, it is essential to thoroughly understand the sow's estrous cycle before considering applying any FTAI protocol. Once the efficacy of the different GnRH agonist used to induce ovulation is known, a protocol can be established that allows the AI to perform at the appropriate time and obtain the best reproductive results.

When talking about FTAI, two types of management can be highlighted. First, estrus detection is used to aid the AI protocol and sows that are not in estrus are not inseminated. Alternatively, FTAI is performed on all sows without detecting estrus. The second protocol can be useful for big farms that assume the risk of inseminating sows who are in anestrus or sows who have had an undetected estrus and, therefore, will not get pregnant.

Obtaining the best reproductive performance after treatment with GnRH agonists requires using good quality semen, sows with good score of body condition (3/5), absence of lameness, and uterine and mammary pathology at weaning. The ovary will then be in the terminal follicular phase and can respond to the induced LH surge. It is essential to comply with the insemination schedule to obtain maximum fertility and prolificacy. Ovulation induction and single insemination will not solve the health and management problems present on the farm; therefore, prior hormonal protocol it is recommended to solve the existing health issues.

For gilts, it is essential that the presence of at least one estrous onsetis controlled prior to synchronization and that the treatment with progestogens is correctly administered. Protocols that reduce the number of inseminations and perform a single post-cervical artificial insemination on gilts have been successfully described (Suárez-Usbeck et al., 2021). However, there is not enough research about the use of new molecules, such as triptorelin.

The main objective of FTAI protocols is to improve productive management on farms. The following are advantages of single fixed-time insemination (Falceto, 2018):

1. Knowing the exact time of insemination.
2. Synchronizing fertilization between different sows to obtain a better homogeneous litter and decrease the number of still born piglets.
3. Spending more time with colostrum intake during the piglet adoption process and increasing the number of piglets weaned per sow.
4. Inseminating all sows with semen from the same boars to potentially decrease birth weight variability between litters.
5. Performing single insemination protocols to save on semen dose cost, which can be invested to improve genetics.

6 | CONCLUSIONS

Improving reproductive efficiency is an important aspect in swine farming. Currently, FTAI with GnRH agonists has proven to be an efficient, successful reproductive protocol that can be implemented in pig farms due to better knowledge of an endocrine system that regulates follicular development and ovulation and increased availability of several GnRH agonists that allow more efficient reproductive swine programs. FTA protocols enable decreases of both number of AI and number of seminal doses needed for pregnancy, accompanied by excellent reproductive performance. FTAI protocols with a single semen dose reduce semen costs in AI and optimize using of selected boars. These techniques reduce estrous detection time and therefore, they ease reproductive management in farms. These protocols also allow a shortening of the time to complete the progeny test of the boar candidate for selection. Finally, they could be combined with new technologies in swine reproduction, such as the use of frozen sexed sperm.

AUTHOR CONTRIBUTIONS

MVF and AS-U developed the concept of the present study and was involved in interpreting data and drafting the manuscript. MTT, RA, AMG, and OM were involved in the search of papers, knowledge transfer, critical discussion of data, and revision of the manuscript. MVF, AS-U, and OM were involved in developing and supervising the project and interpretation of data and drafting and revision of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

María Victoria Falceto https://orcid.org/0000-0001-6964-7011
María Teresa Tejedor https://orcid.org/0000-0001-5026-5144
Olga Mitjana https://orcid.org/0000-0001-9907-2941

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