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Andrés Esteban Suárez Usbeck

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TESIS DOCTORAL

Avances en los protocolos de diagnóstico de la pubertad, inseminación artificial poscervical e inducción de la ovulación en cerdas nulíparas

Andrés Suaréz Usbeck

Zaragoza 2023

Facultad de Veterinaria
Universidad Zaragoza

AVANCES DE LOS PROTOCOLOS DE DIAGNÓSTICO DE LA PUBERTAD, INSEMINACIÓN ARTIFICIAL POSCERVICAL E INDUCCIÓN DE LA OVULACIÓN EN CERDAS NULÍPARAS

NEW DEVELOPMENT IN PROTOCOLS FOR PUBERTAD DIAGNOSIS, POSTCERVICAL ARTIFICIAL INSEMINATION AND OVULATION INDUCTION PROTOCOLS IN GILTS

Autor

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Directoras

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Programa de Doctorado en Medicina y Sanidad Animal

2023

Las Dras. María Victoria Falceto Recio, Profesora Titular del Departamento de Patología Animal de la Universidad de Zaragoza y Olga María Mitjana Nerin, Profesora Contratada Doctora del Departamento de Patología Animal de la Universidad de Zaragoza.

INFORMAN que:

D. Andrés Esteban Suárez Usbeck, licenciado en Veterinaria, ha realizado bajo nuestra dirección durante el periodo comprendido entre 2017 y 2022 los trabajos correspondientes a su Tesis Doctoral titulada **"Avances en los protocolos de diagnóstico de la pubertad, inseminación artificial poscervical e inducción de la ovulación en cerdas nulíparas",** la cual ha sido modificada al título inicial del proyecto de Tesis aprobado por la comisión de Doctorado titulada **"Avances en los protocolos de inseminación artificial poscervical e inducción de la ovulación en cerdas nulíparas".**

Así mismo, certificamos que el material bibliográfico, experiencias y casuística presentados han sido seleccionados y que tanto su elaboración como sus resultados y conclusiones hacen estimar al que suscribe, como directora y codirectora de la Tesis Doctoral, que cumple los requisitos exigidos para optar al grado de Doctor por la Universidad de Zaragoza, por lo que autorizamos su defensa de "Doctor en Medicina y Sanidad Animal", pudiendo ser sometida al tribunal que sea nombrado por la Dirección del Departamento.

Para que conste a los efectos oportunos, emitimos este informe en Zaragoza a 17 de enero de 2023.

Fdo. Dra. María Victoria Falceto Recio

Fdo. Dra. Olga María Mitjana Nerin

A la memoria de mi Abuelo, Gral. José Suárez Rueda

A mi Madre, Grace, a mi Padre, José, a mi Hermano, David y a mi Abuela, Marlene.

“Me enseñaron que el camino del progreso no es rápido ni fácil”.

Marie Curie (1867-1934)

AGRADECIMIENTOS

Esta Tesis doctoral tiene tantos autores como personas han influido en ella. Alguna de ellas lo han hecho de forma directa, aportando ideas, conocimiento o la infraestructura y el material para el desarrollo de la Tesis. Otras de forma indirecta, con su alegría, su comprensión y consejos en los momentos difíciles y su presencia en los momentos felices. Este trabajo es el fruto de una oportunidad que me ofreció mi directora de Tesis, la profesora María Victoria Falceto en la Universidad de Zaragoza y mis padres, al apoyarme en este arduo camino que conlleva realizar un doctorado y la oportunidad de formarme como veterinario investigador en especial en mi rama de la reproducción animal.

En este camino ha habido muchas personas que han participado de manera especial, y a quienes dedico todos los méritos conseguidos durante este proceso. A María Victoria Falceto, mi mentora del Máster de Sanidad y Producción porcina donde nos conocimos y de mi Doctorado, desde el día en que fui a su despacho a preguntarle si tenía un tema de Tesis doctoral. Siempre apoyándome en este camino con sus conocimientos en reproducción porcina, contactos en el sector y su confianza en mí por lo cual estoy agradecido eternamente y que siempre será mi referente en este campo de la veterinaria. Gracias a este apoyo ahora soy docente de una universidad en mí país Ecuador impartiendo los conocimientos y métodos que aprendí de ella y que abre ante mí un camino profesional lleno de oportunidades en la docencia e investigación.

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Publicaciones científicas

Tesis Doctoral

AVANCES EN LOS PROTOCOLOS DE DIAGNÓSTICO DE LA PUBERTAD, INSEMINACIÓN ARTIFICIAL POSCERVICAL E INDUCCIÓN DE LA OVULACIÓN EN CERDAS NULÍPARAS

Memoria de Tesis Doctoral presentada por Andrés SuárezUsbeck

TESIS DOCTORAL POR COMPENDIO DE PUBLICACIONES

Esta Tesis Doctoral está constituida por el compendio de 4 trabajos de investigación publicados en diversas revistas científicas indexadas de carácter internacional, que se presentan a continuación:

Publicación 1: Vela, A., Suárez-Usbeck, A., Lafoz, L., Mitjana, O., Tejedor, MT., Martín, S., López, M., & Falceto, MV. (2022). Determination of puberty in gilts: contrast of diagnostic methods. Porcine Health Management, 8(1), 1-14. https://doi.org/10.1186/s40813-022-00271-0

Publicación 2: Suárez-Usbeck, A., Mitjana, O., Tejedor, MT., Bonastre, C., Moll, D., Coll, J., Ballester, C & Falceto, MV. (2019). Post-cervical compared with cervical insemination in gilts: Reproductive variable assessments. Animal reproduction science, 211, 106207. https://doi.org/10.1016/j.anireprosci.2019.106207

Publicación 3: Falceto, MV., Suárez-Usbeck, Tejedor, MT., Ausejo, R., Garrido, AM., & Mitjana, O. (2023). GnRH agonists: updating fixed-time artificial insemination protocols in sows. Reproduction in Domestic Animals, 58, 571-582. https://doi.org/10.1111/rda.14326

Publicación 4: Suárez-Usbeck, A., Mitjana, O., Tejedor, MT., Bonastre, C., Sistac, J., Ubiergo, A., & Falceto, MV. (2021). Single Fixed-Time Post-Cervical Insemination in Gilts with Buserelin. Animals, 11(6), 1567. https://doi.org/10.3390/ani11061567

Abreviaturas

C.C.A.A: Comunidad autónoma

CIA: Centro de inseminación artificial

cm: Centímetro

eCG: Gonadotropina coriónica equina

EM: Energía metabolizable

EV: Enfermedades venéreas

FSH: Hormona folículo estimulante

g: Gramo

GnRH: Hormona liberadora de gonadotropinas

hCG: Gonadotropina coriónica humana

HHO: Eje hipotalámico-hipofisario-ovárico

IA: Inseminación artificial

IAC: Inseminación artificial cervical

IAPC: Inseminación artificial poscervical

IAIUP: Inseminación artificial intrauterina profunda

IATF: Inseminación artificial a tiempo fijo

Kcal: Kilo caloría

Kg: Kilogramo

LH: Hormona luteinizante

MHz: Megahertz

ml: Mililitro

mm: Milimetro

P4: Progesterona

PB: Proteína bruta

PRE: Prepuberal

PUB: Puberal

µg: Microgramo

UI: Unidades internacionales

Resumen

1

La eficiencia reproductiva en la producción porcina tiene gran importancia, siendo evaluada a través de diferentes parámetros como la fertilidad, la tasa de parto, el número de lechones por cerda y año, el número de lechones destetados, el peso de los lechones al destete, etc. Estos índices pueden estar influenciados por varios factores, tanto ambientales como de manejo, y pueden mejorarse empleando diferentes biotecnologías reproductivas, como es el caso de la ecografía del aparato genital, la determinación de progesterona sanguínea, la inseminación artificial poscervical y los protocolos de inseminación a tiempo fijo con una sola dosis seminal, teniendo como objetivo mejorar el trabajo y el manejo reproductivo en las granjas sin afectar los parámetros de productivos.

La aplicación de nuevos protocolos de inseminación artificial en las cerdas nulíparas está aumentando actualmente, debido a que las estas representan entre el 20 y el 25 % del censo de las granjas, siendo responsables aproximadamente del 13 % de los lechones nacidos. Por lo tanto, se debe de intensificar los estudios realizados en las hembras jóvenes.

El inicio temprano de la pubertad en las hembras nulíparas es necesario para un rendimiento económico adecuado en las granjas porcinas, ya que condiciona el futuro productivo y la longevidad de las cerdas. Por lo tanto, se necesita un método eficaz de diagnóstico de la pubertad. En el **primer artículo** de esta Tesis Doctoral se comparan diferentes procedimientos de diagnóstico para detectar la pubertad en condiciones de granja (características externas de la cerda, espesor de la grasa dorsal, longitud de la vagina y el cérvix, análisis de progesterona en sangre y diagnóstico ultrasonográfico del aparato genital). Concluyendo que tanto la ecografía como la combinación de dos métodos (determinación de progesterona junto con la medida de la longitud de la vagina y el cérvix), pueden ser precisos en el diagnóstico de la pubertad en las cerdas nulíparas, siendo la ecografía realizada por un técnico experto el mejor método de diagnóstico. Los parámetros de precisión se pueden usar como guía para elegir el procedimiento de diagnóstico en la granja, pero también se puede escoger en función de la facilidad de uso, el coste y el efecto estresante en los animales. En este sentido, si el equipo de ultrasonografía esta ya está presente en la

granja, su uso es más económico que el gasto del análisis de la concentración de progesterona. Además, causa menos molestias que la medición de la longitud de vagina-cérvix y toma menos tiempo cuando lo usa un técnico experimentado.

En la actualidad, la mayoría de las granjas de cerdos en todo el mundo utilizan la técnica de inseminación artificial cervical en la primera inseminación de las cerdas nulíparas, mientras que en las multíparas utilizan habitualmente inseminación poscervical. Cuando se usa el método cervical, se aplican entre 2-3 dosis seminales/celo/cerda, con una concentración de 2-4 x 10^9 células espermáticas en un volumen de 70-100 ml. El número de dosis obtenido por eyaculado en este caso está limitado a 20-25 dosis por verraco. En el **segundo artículo** de esta Tesis Doctoral, se ha planteado la inseminación artificial poscervical (IAPC) en la cerda nulípara como una nueva técnica para depositar la dosis seminal en el cuerpo uterino mediante un modelo de catéter y sonda adaptado a la anatomía de las cerdas jóvenes y así, poder obtener las ventajas del método de inseminación poscervical también en nulíparas a nivel de granja comercial. Esta técnica, además de utilizar un número reducido de espermatozoides (1,5-0.5 x 10^9 células espermáticas en un volumen de 30-60 ml), consigue la reducción del reflujo durante la inseminación y la disminución de la duración del procedimiento de inseminación artificial, obteniendo tasas de parto y tamaños de camada similares. La tasa de éxito de paso de sonda hasta el cuerpo uterino fue del 85,7 % (818/958). La frecuencia de reflujo de semen fue menor con la técnica poscervical comparado con la técnica cervical (4,3 *vs* 8,2 %, $p < 0,001$). Hubo valores similares para el diagnóstico positivo de preñez, tasa de parto y prolificidad para ambos grupos ($p>0,05$).

Posteriormente, una vez puesta a punto la técnica de inseminación poscervical en cerdas nulíparas y tras realizar una revisión bibliográfica sobre los agonistas de la GnRH en un **tercer artículo**, quisimos implementar en el **cuarto artículo** de esta Tesis Doctoral la inseminación artificial poscervical a tiempo fijo utilizando una sola dosis seminal tras la inducción de la ovulación con Buserelina, obteniendo resultados productivos similares al uso de dos o más dosis seminales. Esta doble reducción, número de espermatozoides en la dosis seminal poscervical y número de dosis

seminales utilizadas por cerda y celo, conlleva que los verracos en los centros de inseminación artificial puedan mejorar su difusión genética al inseminarse más cerdas con su semen y, además, permitirá en el futuro poder desarrollar nuevas biotecnologías reproductivas porcinas como es el caso de la inseminación artificial con dosis seminales congeladas o encapsuladas de espermatozoides, etc. Es importante destacar que, aunque los objetivos principales de la inseminación artificial poscervical única a tiempo fijo se centran en el progreso genético eficiente producen tasas de fertilidad, tasas de parto y tamaños de camada similares al uso de varias dosis seminales por cerda en celo, ya que no se encontraron diferencias significativas entre grupos ($p > 0,05$). La duración del estro fue significativamente más corta en el grupo Tratamiento ($p < 0,001$). El peso al nacer de los lechones fue mayor en el grupo Tratamiento ($p < 0,001$). La duración del periodo de partos difirió significativamente según la temporada del año ($p < 0,05$); ambos grupos solo difirieron significativamente en primavera ($p = 0,018$), con una menor duración en el grupo Tratamiento. Por todo ello, se recomienda el uso de este nuevo protocolo en cerdas nulíparas.

Summary

2

Reproductive efficiency in swine production is of great importance, being evaluated through different parameters such as fertility, farrowing rate, number of piglets per sow per year, number of piglets weaned, piglet weight at weaning, etc. These indexes can be influenced by several factors, both environmental and management, and can be improved by using different reproductive biotechnologies, such as ultrasound of the genital tract, determination of blood progesterone, postcervical artificial insemination and fixed-time insemination protocols with a single seminal dose, with the objective of improving the work and reproductive management on farms without affecting the productive parameters.

The application of new artificial insemination protocols in nulliparous sows is currently increasing, since they represent between 20 and 25 % of the farm census, being responsible for approximately 13 % of the piglets born. Therefore, studies on young females should be intensified.

The early onset of puberty in nulliparous females is necessary for an adequate economic performance in pig farms, since it conditions the productive future and the longevity of the sows. Therefore, an effective method of puberty diagnosis is needed. In the first article of this Doctoral Thesis, different diagnostic procedures to detect puberty in farm conditions (external characteristics of the sow, thickness of dorsal fat, length of the vagina and cervix, blood progesterone analysis and ultrasonographic diagnosis of the genital tract) are compared. Concluding that both ultrasonography and the combination of two methods (progesterone determination together with the measurement of the length of the vagina and cervix), can be accurate in the diagnosis of puberty in nulliparous sows, with ultrasonography performed by an expert technician being the best diagnostic method. Accuracy parameters can be used as a guide in choosing the on-farm diagnostic procedure but can also be chosen based on ease of use, cost and stressful effect on the animals. In this regard, if ultrasonography equipment is already present on the farm, its use is more economical than the expense of progesterone concentration analysis. In addition, it causes less discomfort than measurement of vagina-cervix length and takes less time when used by an experienced technician.

Currently, most pig farms worldwide use the cervical artificial insemination technique for the first insemination of nulliparous sows, while postcervical insemination is commonly used for multiparous sows. When the cervical method is used, between 2-3 seminal doses/estrus/sow/sow are applied, with a concentration of 2-4 x 109 sperm cells in a volume of 70-100 ml. The number of doses obtained per ejaculate in this case is limited to 20-25 doses per boar. In the second article of this Doctoral Thesis, postcervical artificial insemination (PCAI) in the nulliparous sow has been proposed as a new technique to deposit the seminal dose in the uterine body by means of a catheter and probe model adapted to the anatomy of gilts and thus, to obtain the advantages of the postcervical insemination method also in nulliparous sows at commercial farm level. This technique, besides using a reduced number of spermatozoa (1.5-0.5 x 10^9 sperm cells in a volume of 30-60 ml), achieves the reduction of reflux during insemination and the reduction of the duration of the artificial insemination procedure, obtaining similar farrowing rates and litter sizes. The success rate of probe passage to the uterine body was 85.7 % (818/958). The frequency of semen reflux was lower with the postcervical technique compared to the cervical technique (4.3 vs 8.2 %, $p < 0.001$). There were similar values for positive pregnancy diagnosis, calving rate and prolificacy for both groups ($p>0.05$).

Subsequently, once the technique of postcervical insemination in nulliparous sows had been fine- tuned and after carrying out a literature review on GnRH agonists in a third article, we wanted to implement in the fourth article of this Doctoral Thesis fixed-time postcervical artificial insemination using a single seminal dose after ovulation induction with Buserelin, obtaining productive results similar to the use of two or more seminal doses. This double reduction, number of spermatozoa in the postcervical seminal dose and number of seminal doses used per sow and estrus, means that boars in artificial insemination centres can improve their genetic diffusion by inseminating more sows with their semen and, in addition, it will allow in the future the development of new swine reproductive biotechnologies such as artificial insemination with frozen or encapsulated seminal doses of spermatozoa, etc.

It is important to note that, although the main objectives of single fixed-time postcervical artificial insemination focus on efficient genetic progress, they produce fertility rates, farrowing rates and litter sizes similar to the use of several seminal doses per sow in estrus, since no significant differences were found between groups ($p > 0.05$). Estrus duration was significantly shorter in the Treatment group ($p < 0.001$). Piglet birth weight was higher in the Treatment group ($p < 0.001$). The duration of the farrowing period differed significantly according to the season of the year ($p < 0.05$); both groups only differed significantly in spring ($p = 0.018$), with a shorter duration in the Treatment group. Therefore, the use of this new protocol in nulliparous sows is recommended.

Introducción 3

3.1. LA IMPORTANCIA DE LAS CERDAS NULÍPARAS EN LA PRODUCCIÓN PORCINA

Buena parte de la productividad de una granja porcina depende del correcto manejo de las cerdas nulíparas que han de reemplazar a las cerdas eliminadas (del Castillo Pérez *et al.,*2012). La tasa de reposición media oscila entre el 45 y el 55 %. Porcentajes superiores pueden indicar problemas, ya sean debidos a un inadecuado manejo de las cerdas reproductoras o por la presencia de patologías en la granja y consecuentemente la eliminación prematura de hembras reproductoras antes de alcanzar su máximo potencial genético (Quiles y Hevía, 2007).

El manejo eficiente de las futuras cerdas reproductoras de la granja no solo debe tener en cuenta el número necesario de cerdas de reposición, sino también su calidad, para que de esta manera se pueda asegurar unos excelentes parámetros reproductivos en los primeros partos (Diégues, 2022). Dicho manejo debe ir enfocado a cubrir las necesidades de las cerdas nulíparas, ya que de esa forma vamos a poder obtener el máximo rendimiento durante su vida productiva, con crecimientos de 650 a 850 gramos diarios desde el nacimiento a la primera cubrición. Este indicador es fácil de controlar, ya que crecimientos inferiores darán lugar a presentaciones tardías de la pubertad lo que está correlacionado con un bajo rendimiento reproductivo posterior. También crecimientos por encima de los 850 gramos pueden dar lugar a problemas de aplomos en las reproductoras que repercuten en su rendimiento reproductivo (Castillo y Carrasco, 2019).

Hay que tomar en cuenta que las cerdas nulíparas representan entre el 20 y el 25 % del censo total de cerdas reproductoras de una granja porcina, de ahí que la rentabilidad de la granja dependerá de cómo hagamos el manejo y la preparación de estas cerdas jóvenes (Bortolozzo *et al.,*2015; Hernández-Caravaca *et al.,*2017).

El sistema de selección en las cerdas nulíparas debe basarse en el inicio temprano de la pubertad, la evaluación de las características morfológicas (aparato locomotor, presencia de glándulas mamarias funcionales y número adecuado según la prolificidad de la línea genética y ausencia de hernias u otros problemas físicos),

el correcto control de los primeros celos y una adecuada metodología para la cubrición (Castillo *et al.,*2012).

El manejo de las cerdas nulíparas debe abarcar desde su entrada en la granja hasta el inicio de su segunda gestación. De manera que el obtener una cerda nulípara con un buen desarrollo corporal y con reservas energéticas, permitirá evitar la caída de la prolificidad en el segundo parto y su eliminación temprana como reproductora (Gasa y López-Vergé, 2015).

3.1.1 La pubertad en la cerda

El manejo de las nulíparas y el inicio de la pubertad comienza en el nacimiento y para lograr un resultado exitoso se deberá tener un programa que identifique y elija a las probables cerdas con mejores vidas productivas. Al nacimiento ya se debe confirmar la calidad de esta futura reproductora (más de 14 mamas, salud, crecimiento, etc.) (Menjón *et al.,*2019). Por lo tanto, el peso individual al nacimiento será uno de los principales factores de selección. Las cerdas nulíparas con un bajo peso al nacimiento (<1,1 kg) tendrán la supervivencia y el crecimiento comprometido, lo que los llevará a peores resultados productivos y menor longevidad (Patterson, 2019).

3.1.2.1 Primeras manifestaciones de la pubertad

Una vez la cerda está preparada y seleccionada, pasa de prepuberal a púber y comienza su ciclo productivo con su primer celo. Normalmente alcanzan la pubertad aproximadamente a los 200-210 días de edad; sin embargo, esto vendrá influenciado por muchos factores intrínsecos y extrínsecos como el genotipo, el medio ambiente, el contacto con el verraco o el transporte (Kummer *et al.,*2006). En general, podríamos decir la cerda nulípara estándar en su cubrición tendría 145 kg con 225 días en un segundo celo (Patterson *et al.,*2010).

El inicio de la pubertad a menudo puede coincidir con el transporte de las cerdas nulíparas a la granja destino: si la edad en el momento del transporte está cerca del inicio normal de la pubertad, aproximadamente entre el 25 y el 35 % de

estas mostrarán el celo dentro de la semana siguiente al transporte (Magnabosco *et al.,*2016). Por motivos sanitarios cada día hay más reposición interna o se recibe la reposición externa en grandes lotes muy pocas veces al año. Si tienen la edad y peso corporal adecuados, su primer estro debe ocurrir dentro de un mes después de la llegada de las cerdas a la granja (Menjón *et al.,*2019).

3.1.2.2 Estimulación con el contacto del verraco

El contacto con el verraco será importante para la inducción del primer celo. La edad de la cerda nulípara es muy importante para la eficiencia del verraco como estímulo de la pubertad (Glen, 2017). Por lo que, si el contacto con el verraco se inicia precozmente cuando las cerdas nulíparas tienen 4 meses de edad, la respuesta del estímulo será mínima, incluso pueden llegar a habituarse al estímulo lo que es perjudicial. Por el contrario, cuando la introducción del verraco se retrasa hasta el periodo cercano a la pubertad (6 meses de edad o más), la respuesta también se limita (Patterson *et al.,*2019). Por lo tanto, si el contacto con el verraco se produce a los 5 meses de edad, se optimiza el intervalo desde el primer estímulo con la pubertad, además de producir una sincronización de los celos (Alonso, 2013).

3.1.2 Momento de la primera cubrición

En los últimos años se han conseguido grandes avances en la selección de cerdas nulíparas con respecto a la velocidad de crecimiento y con ello, un mejor desarrollo reproductivo en el inicio precoz de la pubertad (Quiles y Hevia, 2007).

Debido a que existe una correlación negativa entre la velocidad de crecimiento y la longevidad de la cerda reproductora, una incorrecta selección de las futuras reproductoras provocará aumento en la tasa de reposición (Stalder *et al.,*2004). La causa más probable de esto es que, puede que no alcancen una relación grasa/musculo adecuada para desarrollar su pubertad, ocasionando dificultades en las etapas posteriores de la reproducción. De ahí la importancia de conseguir un peso y tamaño corporal uniforme en el momento de la primera cubrición (Coma y Gasa, 2007). Por lo tanto, este aspecto está suponiendo un problema a la hora de la selección de las cerdas nulíparas, ya que alcanzan el peso

ideal para la primera cubrición antes de la pubertad, por lo que existe un gran número de días entre que la cerda joven es considerada como una unidad de la nave de reproductoras y es cubierta por primera vez, siendo estos días improductivos (Quiles y Hevia, 2007).

Este último parámetro puede tenerse en cuenta en la valoración del manejo de las cerdas nulíparas. De tal modo, que uno de los objetivos a este respecto es reducir los costes de mantenimiento, intentando reducir los días improductivos desde que la cerda entra en la nave de control-cubrición hasta que es cubierta por primera vez (Jiménez Viscarra *et al.,*2017).

El conocimiento de las características genéticas de la cerda nulípara es de vital importancia para poder diseñar un correcto plan de manejo con el que conseguir un adecuado desarrollo del aparato reproductor, que permita afrontar con las máximas garantías la primera gestación con una eleada tasa de ovulación y capacidad uterina. Se intentará buscar desde el primer parto la máxima prolificidad, ya que ello nos va a marcar la futura productividad de la granja porcina (Gasa y López-Vergé, 2015).

Durante mucho tiempo se ha considerado como una práctica rutinaria cubrir a las cerdas nulíparas por primera vez a la edad de 6 o 7 meses en el primer celo, lo que implicaba una baja prolificidad en la primera camada, seguida de una tasa de reposición elevada debido a problemas reproductivos después del primer parto (del Castillo Pérez *et al.,*2012).

En los últimos años, junto con la edad y el peso se ha determinado un tercer criterio, a la hora de seleccionar las futuras reproductoras y determinar el momento óptimo de la primera inseminación. Este es, la longitud de la vagina-cérvix, sabiendo que la longitud uterina en la cerda prepúber está correlacionada con la longitud uterina después de la pubertad y, por lo tanto, con la capacidad reproductiva (Martin Rillo *et al.,*2001, Kirkwood *et al.,*2012; Rodrigues, 2018).

Como recomendación, actualmente se aconseja cubrir la cerda en el segundo o tercer celo, teniendo en cuenta tres aspectos variables según su genética

el peso entre los 135 y 150 Kg (dependiendo de la línea genética), el espesor de la grasa dorsal (16-20 mm) en el punto P2 y la edad (> 8 meses) (Nielsen, 2022). Con estos parámetros correctos en el momento de la primera cubrición, la cerda alcanzaría un peso de 180 kg o más en el momento del parto, circunstancia que contrarrestaría los efectos negativos de una pérdida de peso excesiva durante la primera lactación, reduciendo el intervalo destete-cubrición fértil en la segunda gestación.

Una de las principales causas de baja fertilidad o de retorno al celo después de inseminación artificial, se debe a una mala detección del celo en las cerdas nulíparas, enviando al centro de sacrificio cerdas con buenos aparatos reproductores que están con actividad ovárica normal, perdiendo rentabilidad por un incorrecto manejo del diagnóstico del celo (Martinet-Botté *et al.,*2003 y 2004; Tani *et al.,*2016).

3.2. INSEMINACIÓN ARTIFICIAL

La inseminación artificial (IA) en la especie porcina ha permitido que el sector porcino se situé dentro de las primeras posiciones de producción cárnica a nivel mundial. Esta técnica se ha convertido en una pieza clave a la hora de tecnificar y facilitar el trabajo en las granjas, implementar avances en términos de mejora genética de los reproductores de las granjas de núcleos genéticos y el control de la transmisión de enfermedades venéreas (EV) (Gerrits *et al.,*2005; Knox, 2014).

En las últimas décadas se han observado un gran el avance en la biotecnología reproductiva porcina relacionada con las técnicas de conservación seminal (refrigeración y congelación) y en la reducción del número de espermatozoides en las dosis seminales utilizadas en la IA (Compagnoni y Tittarelli, 2019). Esto ha sido posible debido al desarrollo de varias tecnologías en los protocolos de IA como es el caso de la técnica de inseminación artificial poscer- vical (IAPC), depositando el semen en el cuerpo del útero, disminuyendo el volumen y la concentración espermática (Luchetti *et al.,*2016).

La eficiencia reproductiva tiene gran importancia en la producción porcina, siendo analizada por diferentes parámetros como la tasa de fertilidad y de parto, el número de lechones nacidos por cerda/año, el número de lechones destetados/cerda/año, el peso de los lechones al nacimiento y al destete, etc. (Piñeiro *et al.,*2019; Flowers, 2020; Crespo, 2022). Estos índices están influenciados por factores tanto ambientales como de manejo, y se pueden optimizar empleando diferentes protocolos de IA (Lida *et al.,*2015).

El reto de la reproducción porcina es disminuir el número de dosis seminales utilizadas durante los protocolos de IA en cada celo y solo se podrá conseguir profundizando en el conocimiento del inicio, duración y las características del estro en cada granja. Actualmente existen diversas herramientas para la detección del celo como la ecografía ovárica, que pueden ser útiles para conocer la dinámica folicular de las cerdas. Sin embargo, solo la inducción de la ovulación con agonistas de la hormona liberadora de gonadotropinas (GnRH) nos asegurará la programación de una IATF con una sola dosis seminal (Falceto *et al.,*2020)

3.2.1 Antecedentes históricos

La historia de la inseminación artificial porcina se remonta a la década de 1920 en las granjas de la antigua Unión Soviética (Milovanov, 1934). Los avances de la IA continuaron en la siguiente década, con el diseño de los primeros diluyentes para aumentar la vida útil de los espermatozoides almacenados a temperatura ambiente y la elaboración de las primeras dosis seminales comerciales, aunque con poca difusión en las granjas.

En el año de 1957, la IA en el porcino fue reintroducida por Chris Polge en Europa, quien destacó los siguientes beneficios de esta tecnología frente a la monta natural: facilita el uso en la granja de más verracos ayudando al mejoramiento genético, favorece el control de enfermedades, es de fácil implementación independientemente del tamaño de la granja y produce alta rentabilidad económica.

No fue hasta los años setenta y ochenta del siglo pasado, cuando se realizaron las primeras inseminaciones artificiales con semen refrigerado que posteriormente desplazarían a la cubrición o monta natural en la mayoría de las granjas de porcino en varios países. Recién en 1980, la IA se estableció como técnica de reproducción eficaz y viable, pero todavía era necesario seguir mejorando en los protocolos de extracción de semen, almacenamiento seminal e inseminación artificial (Reed, 1982; Das *et al.,*2022).

El uso de semen refrigerado con un promedio de vida útil de 3-7 días utilizado para IA, ha aumentado en los últimos 30 años favoreciendo en muchos aspectos el mejoramiento genético y el control de EV. Actualmente, la IA esta implementada en más del 90 % de las granjas del mundo (Knox, 2016).

En los últimos años, la IA ha contribuido al desarrollo de nuevas biotecnologías relacionadas con la optimización del manejo del estro y la ovulación de la cerda (Falceto *et al.,*2020). Varios factores son los que ayudaron a la implementación de la IA a nivel mundial, entre ellos están: la especialización del sector porcino, la innovación en los catéteres, el mejoramiento genético y la eficiencia de los centros de inseminación artificial (CIA) en la producción de dosis seminales (Fitzgerald *et al.,*2008; Wilson, 2012; Knox, 2016; Cuello *et al.,*2016).

Las primeras investigaciones en campo publicadas utilizando la IAPC fueron realizados por los investigadores Watson y Behan (2002), considerándose hasta la fecha uno de los trabajos más consistentes por el diseño experimental planteado (n= 3.200 cerdas), Sin embargo, es Hancock en 1959 quien describió por primera vez la técnica de IAPC, aunque sus resultados fueron ignorados hasta inicios del siglo XXI.

Desde el principio de la IA, siempre se presentó como uno de los objetivos principales el manejo eficiente del verraco en los CIA (Waberski *et al.,*2019). En la recolección de semen de un verraco adulto se puede obtener un volumen seminal

de 250-300 ml con una concentración de 70-110 x 10^9 de espermatozoides, produciendo entre 20-25 dosis seminales para la técnica IA cervical. Siendo la IAPC una biotecnología más innovadora en las granjas que permite reducir el volumen y la concentración espermática, produciendo más dosis por cada eyaculado (Wilson, 2012; Hernández-Caravaca, 2015).

3.2.1.1 Uso de catéteres en granja

Uno de los desarrollos tecnológicos más importantes de la IA fue el diseño de catéteres que permitieran el paso del semen evitando la contaminación del aparato reproductor de las cerdas (Mellagi et al.,2022).

Los primeros catéteres que se usaron en la IA porcina se fabricaban con materiales reutilizables qué necesitaban de esterilización, equipos especiales y una formación específica. Al cabo de los años, se sustituyeron por catéteres de materiales desechables como el plástico de un solo uso y envasados individualmente. Sin embargo, la gran cantidad de cateres usados diariamente presenta el inconveniente de aumentar la contaminación ambiental considerándose poco sostenible. Por ello, actualmente se está investigando en el diseño de catéteres de material biodegradable con la finalidad de reducir el uso de plástico en las granjas (Fitzgerald et al.,2008; McBride et al.,2019).

A finales de los años 90, se probaron nuevos tipos de catéteres de inseminación artificial porcina y se desarrolló la técnica de la IAPC, teniendo como objetivo mejorar el manejo reproductivo en la granja sin afectar los parámetros de producción. Hasta entonces se había utilizado el catéter de tipo cervical que depositaba el semen en el cérvix de la cerda

Sin el descubrimiento y el desarrollo de la IA, el sector porcino no hubiera alcanzado los actuales niveles de producción de carne de cerdo que se necesitan para satisfacer las necesidades de alimentos de una población mundial en continuo crecimiento (Knox, 2014).

La IA no solo ha facilitado el trabajo en las granjas porcinas, sino que, además, ha favorecido el rápido avance de la mejora genética, mejores índices de producción, bienestar y sanidad animal. A diferencia de la monta natural, esta técnica permite inseminar a un mayor número de cerdas (entre 20 y 60 cerdas por eyaculado), favoreciendo una difusión más rápida del progreso genético en las granjas de todo el mundo y el desarrollo de nuevas biotecnologías en la conservación y transferencia de material genético (Cuello *et al.,*2016; Hernández-Caravaca *et al.,*2017).

3.2.2 Requisitos previos para la inseminación artificial

3.2.2.1 Higiene

La higiene durante la IA es fundamental para prevenir las enfermedades del aparato reproductor de las cerdas (Sánchez-Sánchez, 2006). Antes de la IA es necesario limpiar la vulva con materiales desechables humedecidos en un desinfectante no espermicida para evitar introducir microorganismos en el aparato genital al introducir el catéter durante el protocolo de inseminación (Thibier *et al.,*2000; Althouse *et al.,*2019).

3.2.2.2 Detección del celo

La detección del celo es una de las fases previas a la inseminación para obtener resultados óptimos reproductivos y debe ser lo más precisa posible durante su aplicación en las granjas. Su finalidad es conocer el inicio y el final del periodo del celo (Roca *et al.,*2006). Es una de las tareas más importantes dentro de una granja de reproductoras y debe ser realizada por personal con formación específica en este manejo y ayudado por los machos recela debidamente entrenados en la detección del celo. Los machos deben ser manejables y con alta libido. Idealmente, se debe recelar todos los días, dos veces al día a todas las cerdas de la granja. Se debe evitar inseminar a las cerdas que no presenten el reflejo de inmovilidad durante la recela,

ya que podríamos facilitar una infección del aparato reproductor y la reducción de los parámetros reproductivos de la granja (Knox, 2014).

3.2.2.3 Calidad seminal

Hasta hace pocos años, en el análisis de la calidad seminal para la elaboración de las dosis seminales se realizaba solamente el registro del volumen, una evaluación subjetiva de la motilidad y la concentración espermática medida en cámaras de recuento celular (Hallap *et al.,*2005). En los últimos años, los centros de inseminación artificial (CIA) cuentan con sofisticados programas informáticos asociados a diferentes tipos de microscopios (ópticos, contraste de fases, etc.) que permiten un análisis más preciso y repetible de todos los eyaculados de los verracos (Parrilla *et al.,*2020). De esta manera se lleva a cabo una contrastación seminal completa mediante una evaluación rápida y objetiva de varios parámetros como son: la motilidad, la concentración, la morfología espermática y la integridad del acrosoma pudiéndose complementar el análisis con pruebas más complejas tanto moleculares como genéticas. Estas técnicas permiten obtener dosis seminales con mejor calidad de los espermatozoides (Verstegen *et al.,*2002; Rodríguez *et al.,* 2006; Boe-Hansen y Satake, 2019).

Sin duda, la calidad de las dosis seminales durante la IA es fundamental y cuando es excelente, se puede implementar nuevas técnicas de reducción de la concentración espermática de las dosis seminales. Este es uno de los retos en los que se está trabajando en los CIA de todo el mundo.

En España, el Grupo Operativo ANPSTAND trabaja en un estándar de calidad seminal para los CIA a nivel nacional, que permitirá unificar los procedimientos y criterios en IA y especificar las características mínimas que deben cumplir las dosis elaboradas.

El 47 % de los CIA cuenta con sellos de calidad propios para garantizar los procedimientos de IA (Sánchez, 2021). El objetivo, es crear una única certificación

común que garantice una serie de requisitos mínimos en las instalaciones, el manejo de los animales, la bioseguridad, el bienestar animal, la sanidad e higiene o el medio ambiente.

El desarrollo tecnológico y la investigación en la IA porcina ha sido un factor determinante para desarrollar metodologías pioneras para trabajar en líneas de investigación como: la crioconservación del semen, cariotipado de los animales o en nuevas técnicas de valoración de la calidad seminal.

La IA conlleva numerosas ventajas como un intenso control sanitario, una rápida difusión del progreso genético, la optimización del manejo reproductivo y una disminución de los costeseconómicos de las granjas. Los beneficios comienzan en los centros de inseminación y granjas, que incrementan su competitividad, y repercute en el desarrollo económico de las zonas rurales en las que se asientan y, por ende, al conjunto de la sociedad (ANPS, 2020)

3.2.3 Técnicas de inseminación artificial

Un correcto protocolo y técnica de IA son la clave del éxito reproductivo en una granja porcina. La técnica de IA deposita las células espermáticas directamente en el aparato genital de la cerda mediante el uso de catéteres y dosis seminales, con la finalidad de conseguir una gestación. Existen actualmente tres técnicas de inseminación artificial utilizadas a nivel comercial en todo el mundo (Figura 1): inseminación artificial cervical (IAC), inseminación artificial poscervical (IAPC) e inseminación artificial intrauterina profunda (IAIUP) (Kirkwood y Kauffold, 2015).

Las técnicas intrauterinas IAPC o IAIP requieren la máxima higiene durante el protocolo de inseminación, debido a que depositan el semen directamente en el cuerpo del útero o en los cuernos uterinos, mientras que en la IAC deposita en el cuello uterino, representando este, una importante barrera frente a la entrada de microorganismos patógenos al aparato reproductor de las cerdas (Falceto, 2018).

Existen algunas limitaciones y factores en el uso de las técnicas de IA y la reducción de la concentración de espermatozoides en las dosis seminales que pueden afectar a los parámetros reproductivos (Cane *et al.,*2019). Algunas de ellas son las características anatómicas del cérvix y de los cuernos uterinos representando barreras fisiológicas, junto a que la cerda no posee un eficiente transporte espermático hasta la unión útero-tubárica.

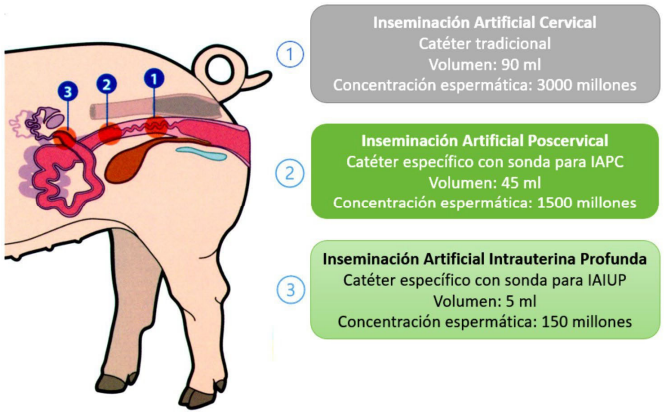

Figura 1. Técnicas reproductivas de inseminación artificial en cerdas (adaptado de Falceto, 2018)**.**

La vida útil de los gametos es otro factor para tomar en cuenta al implementar un protocolo de IA. Además, los espermatozoides están expuestos a la acción fagocitaria de las células polimorfonucleares y a la aparición del reflujo como respuesta fisiológica de eliminación del aparato genital. Por ello es necesario que las condiciones de IA sean la óptimas para no afectar a la fertilidad (Bortolozzo *et al.,*2015).

3.2.3.1 Inseminación artificial cervical

La inseminación artificial cervical (IAC) deposita las células espermáticas en el cuello del útero de la cerda, muy similar a la monta natural en la que el macho eyacula en este sitio (Tabla 1). Su desarrollo e innovación permitió sustituir a la monta natural en las granjas porcinas de cerdo blanco y actualmente se está abriendo camino en la cría del cerdo Ibérico en España (Llamas-López *et al.,*2019).

Es una técnica ampliamente difundida en el sector porcino, que permite obtener buenos resultados productivos debido a las grandes ventajas que tiene en comparación a la monta natural. Existen diferentes tipos de catéteres que varían según los fabricantes, pero también pueden ser específicos para un determinado tipo de cerdas (multíparas o nulíparas). Los catéteres de espiral se introducen en el cérvix rotando en sentido contrario a las agujas del reloj; los de esponja o de multianillas se introducen directamente sin rotación en la porción vaginal del cérvix (Fitzgerald *et al.,*2008, Falceto, 2018).

En la IAC, se utilizan dosis seminales con un volumen aproximado de 80-100 ml, con una concentración de espermatozoides de 3.000 millones de espermatozoides en un tiempo de inseminación de 3-8 minutos. La presencia del macho recela durante la IAC ayuda en este procedimiento provocando contracciones que facilitan el transporte espermático (Speroni, 2014).

Tabla 1. Características de las técnicas reproductivas porcinas monta natural, IAC, IAPC e IAIUP (adaptado de Wilson, 2012 y Falceto, 2018).

	MONTA NATURAL	IAC	IAPC	IAIUP
Volumen (ml)	250-400	70-90	30-45	5
Espermatozoides ($x10^9$)	60-80	2-3	1,5-2	0,5-1
Ubicación del semen	Cérvix	Cérvix	Cuerpo uterino	Cuerno uterino
Duración del proceso	5-10 minutos	3-8 minutos	1-2 minutos	3-5 minutos

Al finalizar la IAC, se suele dejar el catéter insertado durante unos minutos antes de retirarlo del canal vaginal para evitar el reflujo. En el caso de aparecer un abundante reflujo inmediatamente después de la IAC debe repetirse la inseminación. Sin embargo, si el reflujo es escaso y tardío se considera fisiológico (Hernández-Caravaca, 2017).

3.2.3.2 Inseminación artificial poscervical

Desde comienzos del siglo XXI, la IAC está siendo progresivamente desplazada por la inseminación poscervical. Esta técnica de inseminación ha sido determinante para mejorar la difusión genética de los machos reproductores a nivel mundial en el sector porcino. Además, puede disminuir el coste de mano de obra en un 4,66 % y del semen en un 16,18 % (Bolarin, 2016).

La IAPC deposita el semen directamente en el cuerpo del útero a través de una cánula de 72-80 cm de largo que atraviesa el interior de un catéter de inseminación cervical (Ulguim *et al.,*2018). Al no tener que pasar los espermatozoides por la barrera del cuello uterino, esta técnica permite utilizar menos de la mitad del volumen y de la concentración de espermatozoides por cada dosis. Sin embargo, el personal debe estar bien formado en el manejo de la IAPC para evitar lesiones en el cérvix y en el cuerpo del útero que puedan generar infecciones uterinas u otras incidencias reproductivas (Knox, 2016). Por tanto, con un solo eyaculado se puede inseminar el doble de cerdas en comparación con la técnica IAC, sin afectar negativamente al rendimiento productivo de la granja (Ternus *et al.,*2017).

La mayor limitación en la implementación de la IAPC ha sido que no se podía utilizar en cerdas nulíparas (Figura 2) (Garcia-Vásquez *et al.,*2019).

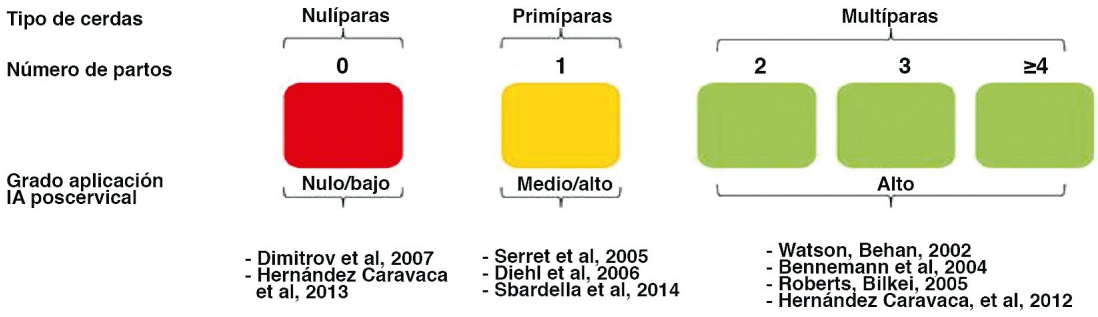

Figura 2. Grado de aplicación de IAPC en diferentes tipos de cerdas (Hernández-Caravaca, 2015).

Para reducir el tiempo de inseminación por cerda durante la IAPC, se recomienda retirar al macho durante el procedimiento para que el cuello uterino de la cerda esté más relajado y la sonda poscervical lo atraviese fácilmente hasta el cuerpo del útero. El tiempo de aplicación de la dosis seminal por cerda es entre 1 y 2 minutos aproximadamente. Si la técnica se realiza correctamente no debería haber reflujo vulvar (Ternus *et al.,*2017; Ausejo *et al.,*2018).

3.2.3.3 Inseminación artificial intrauterina profunda

El primer ensayo que utilizó la IAIUP lo realizó Martínez et al. (2001); el propósito de esta técnica es la deposición del semen en un lugar más cercano a la unión útero tubárica para así disminuir el número de espermatozoides por cada dosis seminal. Este tipo de técnica es muy similar a la de la inseminación poscervical, con la diferencia que el catéter utilizado varía considerablemente en su longitud.

La inseminación intrauterina profunda (IAIUP) deposita el semen en el último tercio de uno de los cuernos uterinos utilizando un catéter cervical y una cánula de una longitud de 148 cm y por esta razón se requiere menos espermatozoides (0,5-1 X 10^9) y volumen de la dosis seminal (5 ml) en comparación a los utilizados en la técnica IAPC (Martínez *et al.,*2010).

Esta técnica no suele utilizarse en las granjas comerciales al ser más complicada. Es necesario contar con personal altamente cualificado y utilizar un catéter más caro. Sin embargo, la aplicación de IAIUP es una herramienta de mucha ayuda para el uso de semen congelado o de espermatozoides que han sido previamente sexados (Bortolozzo *et al.,*2015). También se puede utilizar para técnicas experimentales o en reproductoras de alto valor en granjas de núcleos genéticos.

3.2.3.4 Características morfológicas del cuello uterino

El diseño de nuevos protocolos y equipos de inseminación para la técnica de

IAPC en cerdas nulíparas deben tener en cuenta las variaciones morfológicas del canal cervical, que dependerán de la edad y de las características anatómicas individuales de cada cerda.

La aplicación de IAPC en cerdas multíparas es un protocolo ya establecido en muchas granjas alrededor del mundo, pero su aplicación en hembras nulíparas todavía es un limitante en el ámbito comercial debido a la dificultad de pasar a través de la parte uterina del cérvix. Se plantea como hipótesis que el aumento en el número de la paridad modifica la morfología del canal cervical permitiendo pasar mejor las cánulas a través del cérvix y, por tanto, realizar con mayor facilidad la IAPC en las cerdas multíparas que en las nulíparas (Rodríguez-Antolín *et al.,* 2012).

En el estudio morfológico del cérvix realizado por García-Vázquez *et al* (2019a), demostraron que el cérvix es más largo en las cerdas multíparas que en las cerdas nulíparas (4 cm más en la parte uterina y 6 cm más en la parte vaginal). Con respecto a la sección transversal del cérvix fueron mayores en la parte uterina de las multíparas que en las nulíparas: área (4,07 *vs* 2,46 cm^2), el perímetro (8,50 *vs* 6,28 cm) y el grosor total (10,79 *vs* 8,35 cm), pero no hubo diferencias significativas en la parte vaginal entre los dos tipos de cerdas.

El contenido de tejido analizado en las secciones transversales histológicas también mostró diferencias entre las hembras nulíparas y multíparas, con un mayor contenido de tejido conectivo (12,37 vs 10,79 mm) y una menor cantidad de fibras musculares (8,35 vs 11,50 mm). Finalmente, los moldes de silicona del canal cervical revelaron diferencias entre los dos grupos en el tamaño y la forma de los pliegues durante su trayectoria (figura 3).

La edad y la paridad determinan cambios importantes en el tamaño, la estructura y el contenido del tejido de la pared del cuello uterino, así como en la morfología del canal cervical, que puede ser responsable de los diferentes niveles de rendimiento de la IAPC en las distintas fases de crecimiento de las cerdas. Por lo tanto, en el diseño futuro de las estrategias de IA y de los catéteres se deben tener en cuenta las características morfológicas del canal cervical, que dependerán de la

edad y la paridad de las cerdas en sus distintas etapas de crecimiento (Winn et al.,1993; Sbardella *et al.*,2014).

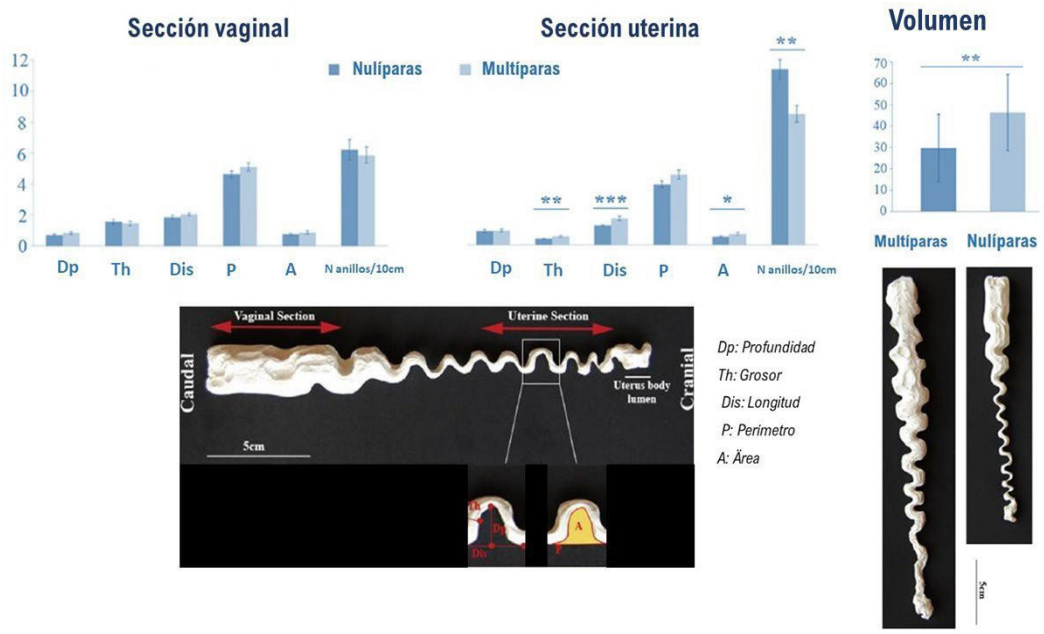

Figura 3. Diferencias morfométricas y anatómicas en el cérvix de cerdas multíparas y nulíparas adaptado de García- Vázquez et al., 2019ª

3.2.3.5 Inseminación artificial en cerdas nulíparas

El principal objetivo de la IA es depositar suficientes espermatozoides viables en el lugar apropiado del aparato genital de las cerdas (cuello uterino, cuerpo del útero o en los cuernos uterinos) en el momento óptimo con respecto a la ovulación. Para las cerdas nulíparas, los protocolos tradicionales de IA recomiendan el depósito de 2-3 dosis seminales en el cuello uterino cada 12–24 horas después de la detección del celo (2–4 x 10^9 espermatozoides y un volumen de 60–100 ml por dosis) (Kirkwood y Kauffold, 2015).

Las razones de realizar varias IAC se sustentan en la breve viabilidad tanto de los ovocitos como de los espermatozoides. El tracto reproductivo característico de estas cerdas y la dificultad de predecir el inicio de la ovulación durante el celo

son los factores más importantes en la inseminación artificial en cerdas nulíparas (Belstra *et al.,*2004).

La técnica de inseminación artificial poscervical (IAPC) fue propuesta como una nueva técnica alternativa a la inseminación artificial cervical (IAC) en las cerdas nulíparas, debido no solo al sitio de deposición del semen, sino también en las ventajas que trae en la reducción del tiempo de inseminación (Bortolozzo *et al.,*2015; García-Vázquez *et al.,*2019b).

La IATF implica la aplicación de una sola dosis de semen dentro de un período de 0 a 24 horas antes de la ovulación (Baroncello *et al.,*2017). La comprensión de los mecanismos de regulación del desarrollo folicular y de la ovulación han permitido desarrollar nuevas tecnologías aplicadas en la inducción de la ovulación en cerdas nulíparas (De Rensis *et al.,*2016). En cerdas nulíparas la duración del celo y la duración del intervalo entre el inicio del estro y la ovulación son muy variables; por lo tanto, uno de los objetivos es controlar el momento de la ovulación para establecer mejores programas de IA (Brussow *et al.,*2009; Baroncello *et al.,*2017).

Los agonistas de la hormona GnRH, se han utilizado de manera eficiente para inducir la ovulación en cerdas nulíparas con excelentes resultados, pero siempre utilizando la IAC (Kirkwood y Kauffold, 2015).

Los protocolos más simples con solo la aplicación hormonal de agonistas de la GnRH mostraron una excelente eficiencia en la inducción de la ovulación y con ello la reducción del número de inseminaciones por celo (Knox *et al.,*2011; Quirino *et al.,*2019). Se necesita realizar más investigaciones para el desarrollo de nuevos protocolos de IAPC e IATF en cerdas nulíparas para mejorar el desempeño reproductivo de las granjas porcinas.

3.2.4 Momento óptimo para realizar la inseminación artificial

Los mejores rendimientos de fertilidad y prolificidad se producen durante las IA realizadas horas previas a la ovulación. Los espermatozoides se pueden mantener

fecundantes durante 24 horas y los ovocitos durante 6-8 horas (Driancourt *et al.,*2013). Si se insemina antes o después de ese momento, los gametos pierden su capacidad fecundante generando repeticiones de celo o pérdidas embrionarias y reducción del tamaño de la camada.

El celo de la cerda dura entre 1 y 3 días, realizando habitualmente varias inseminaciones durante el mismo (2,4 IA/celo), con un intervalo de 12-24 horas entre cada IA, desde que la cerda muestra el reflejo de inmovilidad ante la presencia del macho recela hasta el final del estro en el que este reflejo desaparece.

El objetivo de mejorar este manejo reproductivo es intentar que una de esas IA se aproxime al momento de la ovulación que ocurre dentro de 30 a 40 horas después de iniciado el celo y la liberación de los ovocitos dura alrededor de 3 a 7 horas. Por esta razón una correcta IA se debe realizar entre las 20-30 horas después de la detección del inicio de celo, ya que coincide con el período óptimo de supervivencia de los espermatozoides (Mazzarri, 1984).

Cabe mencionar que las inseminaciones tardías realizadas en granjas en la que la detección del celo no es correcta pueden dar lugar a descargas vulvares, repeticiones de celo e infertilidad, por lo que, se recomienda realizar correctamente la detección del celo y la técnica de IA en las cerdas (Soede, 1998).

3.2.4.1 Estimación del momento de la ovulación

Para disminuir el número de inseminaciones durante el celo de la cerda es necesario conocer el momento de la ovulación. Pese a que se han estudiado muchos métodos para identificar el momento de la ovulación, hasta la fecha no se ha encontrado una solución que sea de fácil implementación por el personal de las granjas (Knox *et al.,*2017). Las técnicas actuales están basadas en diferentes métodos entre los que se destacan los niveles hormonales de hormona luteinizante (LH), la citología vaginal, la resistividad del moco vaginal, la temperatura de la vulva o la ecografía ovárica (Falceto, 2016; Zhang *et al.,*2019; White *et al.,*2020; De la Cruz-Vigo *et al.,*2022; Matos *et al.,*2022; Knox, 2022).

Una técnica utilizada actualmente en las granjas para estimar el momento de la ovulación es determinar la duración media del celo en las cerdas multíparas. Para ello se debe anotar la duración del celo de las cerdas de una granja durante un periodo de tiempo (Falceto, 2018) y calcular que la ovulación ocurre una vez ha transcurrido el 70 % del tiempo del celo (Kirkwood y Kauffold, 2015).

Se recomienda realizar la IA entre las 8-12 horas antes de la ovulación estimada. El fundamento es que el momento de inicio del celo y su duración, suelen ser semejantes en las hembras de un mismo grupo, como respuesta a los factores comunes que comparten como son la genética, alimentación, iluminación, temperatura, humedad, ventilación, bienestar, manejo de los machos recela, etc. (Knox, 2016). No obstante, no hay que olvidar que la estimación está basada en una información que puede ser subjetiva dependiendo de la formación de los operarios de cada granja.

El diagnostico mediante ecografía ovárica es sin duda alguna el método más preciso, pero presenta la desventaja de que requiere personal altamente cualificado y mucho tiempo de evaluación de las cerdas en la granja (Kauffold y Althouse, 2007; Pelthoniemi *et al.,*2019). Los actuales equipos de ecografía abdominal permiten comprobar si las pautas habituales de inseminación utilizadas en cada granja porcina son adecuadas al momento de la ovulación.

El seguimiento del crecimiento folicular ovárico se realiza cada 6-12 horas desde el inicio del celo hasta que los folículos alcanzan el tamaño preovulatorio. La ovulación se detecta al desaparecer del ovario los folículos preovulatorios y aparecer los cuerpos *rubrum* (Koketsu *et al.,*2017).

3.3 INDUCCIÓN DE LA OVULACIÓN EN LAS CERDAS

Las hormonas reproductivas utilizadas para el control del desarrollo folicular y la inducción de la ovulación han estado disponibles en la industria porcina durante los últimos 60 años, sin embargo, apenas han sido utilizadas, con excepción de los protocolos de tratamiento de determinados casos de anestro (Hühn *et al.,*1996).

Algunas de las razones de su escaso uso en el pasado fueron su elevado coste y la escasa aplicación práctica a nivel de granja (Knox, 2015; Hayden, 2008).

En la actualidad los agonistas de la GnRH tienen gran interés para la industria porcina. Varios son los estudios que se disponen para establecer diferentes protocolos de inseminación artificial a tiempo fijo (IATF) en cerdas multíparas (Von Kaufmann y Holtz, 1982; Brüssow *et al.,*1990; knox *et al.,*2003; Martinat-Botté *et al.,*2010). Su aplicación en los protocolos de IA en las granjas puede ayudar a aumentar el mejoramiento genético y la eficiencia de la producción porcina (Baroncello *et al.,*2016; Pearodwong *et al.,*2019; Lopes *et al.,*2020; Rodríguez *et al.,*2020).

3.3.1 Historia del desarrollo de las hormonas para la inducción de la ovulación

El desarrollo de las primeras hormonas reproductivas para la inducción de la ovulación y para controlar el estro comenzó en el siglo XX cuando se descubrió la existencia de las lesiones hipofisarias producia atrofia del aparato genital, identificando así, el eje hipotálamico- hipofisario-ovárico (HHO). Louria y Rosenzweig (1982), demostraron la estimulación gonadal con muestras de orina obtenida de mujeres gestantes, por la presencia de gonadotropina coriónica humana (hCG).

Fevold en 1931, proporcionó la primera evidencia de la existencia de dos gonadotropinas hipofisarias, la hormona folículo estimulante (FSH) y la hormona luteinizante (LH) (Knox, 2015; Hayden, 2008). En los siguientes años, la estimulación ovárica con gonadotropinas exógenas: gonadotropina coriónica equina (eCG) y gonadotropina coriónica humana (hCG), se utilizaron para indución de la ovulación, aunque la formación de anticuerpos y la poca bioseguridad de las moléculas aisladas en aquella época limitaron su uso comercial en el campo de la medicina veterinaria (Hayden, 2008).

Tanabe *et al* (1949), fueron los primeros en utilizar la hCG para estimular el desarrollo folicular e inducir la ovulación en las cerdas (Brüssow y Wähner, 2011). Estos descubrimientos fueron el pilar del desarrollo de protocolos de sincronización del celo e inducción de la ovulación, como los realizados por Polge y Day (1969), usando metaliburo para restringir el crecimiento de los folículos seguido del tratamiento con eCG para estimular el desarrollo folicular junto a la hCG para la inducción de la ovulación.

En la década de 1960, la biotecnología era lo suficientemente avanzada para extraer la FSH y LH de muestras de la hipófisis de varias especies animales, aunque aún existían problemas en la bioseguridad y la pureza de las hormonas, produciendo una pobre eficiencia farmacocinética (Hayden *et al.,*1999). Pese a todo, se empezaron a utilizar de forma rutinaria en las cerdas. Las gonadotropinas exógenas administradas en los últimos años a cerdas nulíparas y multíparas para el control del ciclo estral son la eCG, una glicoproteína con actividad análoga a la FSH y LH, y la hCG, con un efecto similar a la LH (Farmer y Papkoff, 1979; Kirwood, 1999).

En 1971, fue aislado el decapéptido de la GnRH hipotalámica, permitiendo identificar y estudiar regiones específicas de la adenohipófisis y determinar la activación y la estabilidad de su unión al receptor hipofisario de la GnRH (Hayden, 2008; Schally, 1999; García, 2004). Posteriormente, uno de los primeros agonistas de la GnRH fue la gonadorelina, tomando la iniciativa de su uso en cerdas a finales de los años 1970 (Brüssow y Bergfeld 1979).

El descubrimiento de la estructura y síntesis del agonista de la GnRH llevó a que se convirtiera en un potencial sustituto de las gonadotropinas en la estimulación del crecimiento folicular y la inducción de la ovulación (Brüssow *et al.,*1996; López, 2009). Los agonistas de la GnRH tienen una mayor afinidad por los receptores de la adenohipófisis, permaneciendo unidos por más tiempo y estimulando la secreción de FSH y LH, lo que los hace más eficientes que la propia

GnRH endógena (Fries *et al.,*2010). Al inducir la ovulación en las cerdas permiten realizar la IATF en un momento cercano a la ovulación (Kirkwood y Kauffold, 2015).

Durante los años posteriores se han desarrollado diferentes moléculas de agonistas de la GnRH con el fin de tener un mejor control sobre el desarrollo folicular, el momento y la calidad de la ovulación, además, de mejorar los protocolos reproductivos en granjas comerciales. Los siguientes agonistas de la GnRH han sido probados para estimular la secreción de LH e inducir la ovulación en cerdas: gonadorelina (Brüssow *et al.,*1990; 1996), lecirelina (Baruselli *et al.,*2001);peforelina (Hunter *et al.,*2004; Brüssow *et al.,*2010; de Jong *et al.,*2017), goserelina (Brüssow *et al.,*2007), buserelina (Martinat-Botté *et al.,*2010; Driancourt *et al.,*2013) y triptorelina (Knox, 2011; 2017).

El uso de agonistas de la GnRH para inducir la liberación de LH tiene varias ventajas en comparación con el tratamiento de hCG. Mientras que la hCG actúa directamente sobre los receptores ováricos, los agonistas de la GnRH estimulan la liberación de LH por la hipófisis, que después se dirige al ovario para ayudar en el proceso de la ovulación (Carlson, 2019). Los resultados pueden variar debido a factores del desarrollo del folículo en relación con los protocolos de inducción, el tipo de agonista, las dosis utilizadas y el momento de administración (Knox, 2015; Pearodwong *et al.,*2019).

La buserelina es un agonista de la GnRH que se utiliza actualmente en cerdas (Figura 4). Estudios llevados a cabo por los equipos de Von Kaufmann y Holtz (1982) y Martinat-Botté *et al* (2010) demostraron que la administración de 10 µg de buserelina induce eficientemente ovulación en cerdas nulíparas pretratadas con eCG.

Actualmente, la recomendación para cerdas multíparas es una inyección intramuscular de 10 µg de buserelina 83-89 horas después del destete y una inseminación única 30 a 33 horas después, ocurriendo la ovulación 42 ± 2 horas tras

la administración de buserelina (Falceto *et al.,*2014; Baroncello *et al.,*2017; Pearodwong *et al.,*2019; Lopes *et al.,*2020).

Para las cerdas nulíparas, Driancourt *et al* (2013) explica que el momento de la ovulación se produce a las 35-41 horas postratamiento con buserelina, administrada 86 ± 3 horas después del destete.

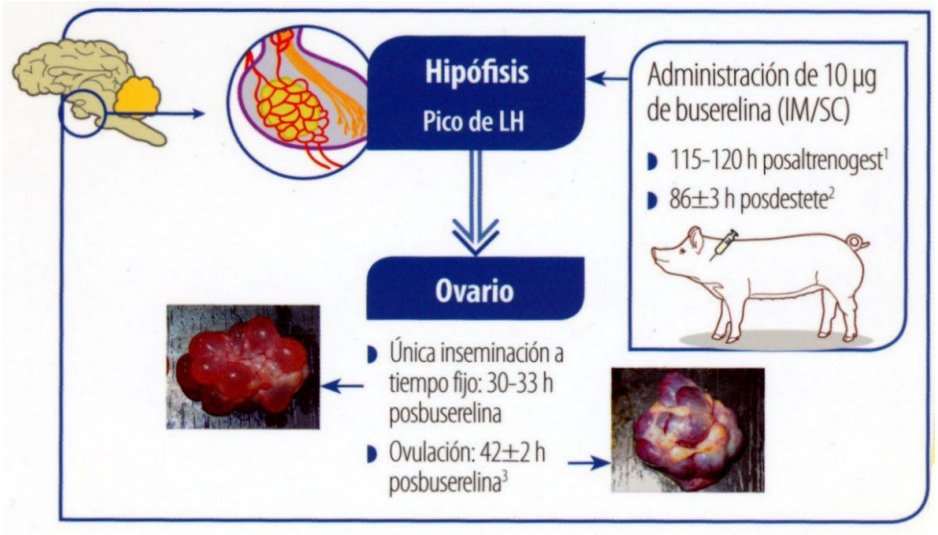

Figura 4. Protocolo de inducción de la ovulación e inseminación con buserelina (Falceto, 2018).

Martinat-Botte *et al* (2010) realizó un trabajo en cerdas nulíparas inducidas con 10µg de buserelina administrada 115-120 horas después del último día de tratamiento de sincronización del celo con altrenogest e inseminado dos veces 30-33 horas después del tratamiento con buserelina, obteniendo un 84,5 % de tasa de partos.

3.3.2 Momento óptimo para la inducción de la ovulación

Para realizar correctamente la inducción de la ovulación, se debe determinar cuidadosamente tres momentos: la administración del agonista de la GnRH, cuando

las cerdas muestran los signos de estro y la hora de la inseminación. Es esencial comprender a fondo el ciclo estral de las cerdas antes de considerar la aplicación de cualquier protocolo hormonal para la IATF. Una vez conocida la eficacia de las diferentes hormonas agonistas de la GnRH utilizadas para inducir la ovulación, se puede establecer un protocolo para que la IA se realice en el momento adecuado y obtener los mejores resultados reproductivos.

Basándonos en la vida útil de los gametos (8-12 h y 24 h; ovocitos y espermatozoides respectivamente), el momento óptimo para cubrir a la cerda para obtener excelentes índices productivos es realizar la IA 24 horas antes de la ovulación (Cassar *et al.,*2005).

Sin embargo, otro factor importante es que existe una gran variación individual del intervalo desde el destete a la aparición del celo y de propia duración del estro y, en consecuencia, también del inicio del celo a la ovulación (De Rensis *et al.,*2003; Cassar *et al.,*2005), presentando un reto el determinar el momento óptimo de la ovulación. La variación en el inicio del estro y la ovulación podría estar asociado con la heterogeneidad del desarrollo de los folículos y su respuesta al pico de LH (Nissen *et al.,*1997; Knox, 2015

El uso de agonistas de la GnRH para la inducción de la ovulación permite predecir con mayor exactitud el momento de la ovulación para realizar correctamente las IA (Cassar, 2005). La elección del momento correcto de administración de los agonistas de la GnRH para la inducción de la ovulación puede asegurar el éxito de la IATF, ya que el nivel de LH inducido por la administración de un agonista de la GnRH es bastante similar al pico de LH inducido por la GnRH endógena (Degenstein *et al.,*2008; Driancourt *et al.,*2013).

Los fallos del protocolo de inducción de la ovulación con los agonistas de la GnRH se deben a que es posible que el pico de LH no llegue al valor umbral necesario para activar la cascada ovulatoria o que la señal del pico de LH no se produzca en el momento esperado (Castagna *et al.,*2004), provocando pérdidas embrionarias por

realizar inseminaciones durante el estro tardío (Rozeboom *et al.,*1997).

Sin embargo, en la mayoría de las ocasiones, los fallos reproductivos con técnicas de IATF están relacionadas con la aplicación de los agonistas de la GnRH en cerdas que no se encuentran en la fase de proestro tras la administración del progestágeno o tras el destete. La razón es que son impúberes, están en anestro posdestete o han tenido un celo no detectado en la sala de maternidad antes del destete y se encuentran en la fase de diestro con presencia de cuerpos lúteos (Falceto, 2018).

3.3.3 Protocolos actuales de IATF con agonistas de la GnRh

Un protocolo rutinario para la estimulación del celo en cerdas, sobre todo en el verano, es la inyección de la combinación de 400 UI de eCG y 200 UI de hCG, demostrando una eficacia en la inducción de un celo fértil después del destete o de la sincronización del celo con altrenogest, pero sin provocar la inducción de la ovulación (Kirkwood, 1999). Además, al estimular un inicio más temprano del celo, el intervalo estro-ovulación es más largo, lo que hace que la predicción del momento de la ovulación sea incluso más difícil que en un celo natural (Knox *et al.,*2001; Estienne *et al.,*2001). Otro inconveniente del uso de la combinación de eCG y hCG en dosis altas (700 y 350 UI, respectivamente) es el riesgo de generar quistes ováricos, luteinización de los folículos y degeneración ovárica quística (Brüssow y Whaner, 2011).

Los requisitos para obtener los mejores resultados tras el tratamiento con agonistas de la GnRH son utilizar semen de buena calidad y que la hembra presente buena condición corporal no esté coja ni presente patologías uterinas y se encuentre en la fase de proestro. Entonces, el ovario estará en fase folicular terminal, pudiendo responder al pico inducido de LH (Falceto, 2018). Posteriormente, es imprescindible cumplir el horario de inseminación para obtener la máxima fertilidad y prolificidad (Knox, 2015).

En el caso de las hembras nulíparas, además, es fundamental que se haya

controlado la presencia de un celo previo a la sincronización y que se administre correctamente el tratamiento con progestágenos. Sin embargo, hasta ahora, no hay estudios suficientes con el uso de nuevas moléculas como la buserelina y la triptorelina (Knox, 2017).

Al hablar de IATF, se pueden destacar dos tipos de manejo: uno en el que se utiliza la detección de celo como ayuda al protocolo de IA y no se inseminan las cerdas que no están en celo; otro, en el que se realiza en todas las cerdas la IATF sin detección del celo. En este último protocolo, puede ser útil en granjas muy grandes, asumiendo el riesgo de inseminar cerdas en anestro o cerdas que han tenido un celo no detectado en la maternidad y que por tanto no van a quedar preñadas.

No debemos olvidar que el objetivo principal de los protocolos de IATF no es mejorar los resultados productivos, si no gestionar mejor el trabajo en la granja. Las ventajas de la inseminación única a tiempo fijo son las siguientes (Falceto, 2018):

- Conocer el día y la hora exacta de la inseminación, pudiendo dedicar el personal más preparado a esta misión.
- Obtener mayor agrupamiento entre los partos (al sincronizar la fecundación entre las diferentes hembras del lote) permite realizar una mejor atención y una disminución del número de lechones nacidos muertos. Al dedicar más tiempo a la toma del calostro y al proceso de las adopciones de los lechones, podría aumentar el número de lechones destetados por cerda.
- Inseminar todas las cerdas del lote con el semen del mismo padre, puede disminuir la variabilidad de peso al nacimiento entre las camadas.
- Al ser una única inseminación, el ahorro económico en dosis seminales se puede invertir en la mejora la genética. La utilización de dosis homoespérmicas de los machos es importante en la diseminación del progreso genético en las granjas.

Justificación y objetivos

4

Esta Tesis doctoral ha sido desarrollada en el Área de Reproducción y Obstetricia del Departamento de Patología Animal de la Facultad de Veterinaria de la Universidad de Zaragoza, centro de referencia nacional e internacional en control de la reproducción y diagnóstico de patología reproductiva porcina.

El trabajo de investigación presentado se ha llevado a cabo en las regiones de Aragón y Cataluña, ambas destacadas como punteras en la producción porcina. Cabe destacar que España es el segundo país europeo en producción porcina con más de 32 millones de cerdos y más de 4,6 millones de Tm de carne; y el cuarto del mundo productor de carne de cerdo (Álvarez, 2020).

En este contexto las cerdas nulíparas son un punto clave para mejorar el progreso genético y el potencial productivo, al mismo tiempo que la estabilización y mejora del estado sanitario de la granja. Este grupo de cerdas son consideradas el corazón de la granja ya que cuanto mejores son, mejor será el rendimiento productivo (Foxcroft, 2001). De esta manera tener un numero correcto de cerdas nulíparas cubiertas es el parámetro más influyente para mantener constante la producción (Carrasco y Castillo, 2015). Sin embargo, una reposición de más del 50 %, de las cerdas reproductoras va a repercutir en la estructura censal de la granja con un descenso en los parámetros reproductivos como el número de lechones destetados totales.

A pesar de que hay numerosos estudios actuales tanto en nuevas biotecnologías aplicadas a la reproducción de las cerdas multíparas como en la inseminación a tiempo fijo (Quirino *et al*; 2019; Gianluppi *et al*; 2021; Tummaruk *et al*; 2022) o en la conservación seminal (Pavaneli *et al*; 2020; García, 2021; Ausejo *et al*; 2022; Sánchez-Sánchez *et al*; 2022), apenas hay estudios novedosos aplicados a las cerdas nulíparas.

La adaptación de las futuras cerdas reproductoras de la granja afectará no solo a su primer parto, sino también a los partos restantes durante toda su vida productiva (Edwards, 1997; Piñeiro y Kosketsu, 2015), por lo que la primera cubrición

es uno de los puntos clave en los que debemos concentrar nuestros esfuerzos en el manejo de las cerdas nulíparas (Stalder *et al*; 2003; Engblom *et al*; 2016; Li *et al*; 2018).

Una correcta aclimatación permitirá mejorar la producción, en torno a un lechón más, si cubrimos a las cerdas nulíparas con un peso adecuado en su segundo o tercer celo (dependiendo de la genética) después de la sincronización del celo con 20 mg de altrenogest por 18 días y con dos o tres inseminaciones por celo (Menjón, 2020; Marcial *et al*; 2020; Martínez *et al*; 2021

Un protocolo que permite evaluar la adaptación productiva y la aclimatación de las nulíparas, a la vez que su bienestar animal es el cálculo de la tasa de retención, es decir, el porcentaje de cerdas que alcanzan el tercer parto respecto a las cerdas que entraron en la granja. Así, se consideraría favorable un valor mayor del 75 % (Stancic *et al*; 2012).

La tasa de eliminación de nulíparas que no han llegado a parir puede llegar a ser muy elevada (Kummer *et al*; 2006) a pesar del gran coste tanto de los animales como de su alimentación y mantenimiento en la granja. Las causas de eliminación mayoritariamente son debidas a un fallo reproductivo como por ejemplo ausencia de celos, infertilidad, etc. (Kumar y Singh, 2015).

El trabajo de investigación de esta Tesis doctoral se ha enfocado en la aplicación de nuevas biotecnologías en varios momentos claves del manejo reproductivo de las cerdas nulíparas. En primer lugar, se ha investigado en la evaluación y comparación de distintas herramientas de diagnóstico de la pubertad en condiciones de granja comercial. La ausencia de pubertad o un mal diagnostico suponen la eliminación de una cerda nulípara de la granja (Articulo 1). Estudios previos demostraron que entre un 40 a 60 % de las cerdas nulíparas eliminadas por anestro eran cíclicas probablemente resultado de una inadecuada detección de la pubertad (Falceto, 2015, Tummaruk *et al*; 2015; Patterson y Foxcroft, 2019).

En segundo lugar, se ha evaluado la técnica de inseminación poscervical también a nivel de una granja comercial, comparado con el protocolo de Inseminación convencional (cervical) usado habitualmente en las cerdas nulíparas. La técnica de inseminación poscervical en las cerdas multíparas, como se ha descrito previamente (Watson y Behan, 2002; Bennemann *et al*; 2004; Serret *et al*; 2005; Roca *et al*; 2011; Ausejo *et al*; 2017; Ulguim *et al*; 2018; Llamas-López *et al*; 2019) se usa de manera rutinaria en todo el mundo. Las ventajas del uso de esta técnica son importantes, ya que se reducen la concentración espermática necesaria siendo más eficiente el uso del verraco (Serret *et al*; 2005) y la aplicación de la dosis seminal al tener menos volumen es más rápida. El hándicap en el caso de las cerdas nulíparas es el menor tamaño de su cérvix (García- Vásquez *et al*; 2015) por lo que en nuestra investigación se utilizara un nuevo catéter especialmente diseñado para nulíparas (Articulo 2).

En tercer lugar, se ha realizado una revisión bibliográfica que abordará el uso de los distintos agonistas de la GnRH y su importancia en la inducción de la ovulación de las cerdas para establecer nuevos protocolos de IATF en cerdas nulíparas (Articulo 3). Y finalmente se llevó a cabo un experimento del efecto de la buserelina como inductor de la ovulación y analizando los resultados reproductivos y productivos obtenidos con una sola IATF frente a una doble inseminación sin inducción hormonal, realizadas mediante la técnica IAPC validada en el artículo 2. El uso conjunto de la inseminación poscervical junto con la IATP en condiciones de granja comercial se ha validado en cerdas multíparas (Wilson, 2012; Hernández-Caravaca *et al*; 2015) siendo novedoso su uso en nulíparas (Articulo 4).

El desarrollo de estas nuevas técnicas permitirá obtener mejores índices reproductivos y prevenir patologías que puedan afectar a los parámetros productivos de las cerdas nulíparas, y poder ser utilizados en las futuras biotecnologías reproductivas más avanzadas como la inseminación con semen congelado, encapsulado y sexado.

El principal objetivo de esta Tesis doctoral es investigar la aplicación de las nuevas biotecnologías reproductivas en las cerdas nulíparas. Para ello, se propusieron los siguientes objetivos específicos:

- **Objetivo 1:** Evaluar la eficacia de los diferentes métodos de diagnóstico de la pubertad en las cerdas nulíparas en condiciones de granja porcina comercial (Artículo 1).
- **Objetivo 2:** Comparar en condiciones de campo la utilización de las técnicas de IAC y la IAPC realizada mediante catéteres específicamente diseñados para las cerdas nulíparas sobre las variables de fertilidad, tasa de parto y prolificidad, así como las posibles complicaciones y tiempo de inseminación (Artículo 2).
- **Objetivo 3:** Realizar una revisión bibliográfica de los agonistas de la GnRH y su importancia en la inducción de la ovulación de las cerdas para establecer nuevos protocolos de IATF en nulíparas (Artículo 3).
- **Objetivo 4:** Determinar en las cerdas nulíparas de una granja comercial el efecto de la buserelina como inductor de la ovulación y analizar los resultados reproductivos y productivos obtenidos con una sola IATF frente a una doble inseminación sin inducción hormonal, realizadas mediante la técnica IAPC validada en el objetivo 2 (Artículo 4).

Material y métodos

5

El trabajo experimental de la Tesis doctoral está enfocado en dos experimentos en cerdas nulíparas.

El primer experimento se basa en los métodos de diagnóstico precoz de la pubertad en las cerditas de la granja (artículo 1).

El segundo experimento tiene dos ensayos basados en la técnica de inseminación artificial poscervical aplicada a la primera cubrición de las cerditas (artículos 2 y 4).

En el primer ensayo del segundo experimento se pone a punto la técnica de inseminación artificial poscervical en nulíparas y se comparan sus resultados con la técnica habitualmente utilizada en las granjas (inseminación intracervical).

El segundo ensayo del segundo experimento se basa en la inducción hormonal de la ovulación con un agonista de la GnRH previamente a la primera inseminación de la cerdita realizada a tiempo fijo con una sola dosis seminal comparado con dos inseminaciones realizadas en cerditas no tratadas hormonalmente (artículo 4).

5.1 DECLARACIÓN ÉTICA

Esta Tesis doctoral siguió los requerimientos y políticas de las siguientes pautas y normativas sobre el bienestar animal y ética del uso de animales para experimentación científica. Se cumplieron las directrices ARRIVE (Kilkeny et al., 2010), la Directiva 2008/120/CE del Consejo relativa a las normas mínimas estandar para la protección de cerdos y la Directiva 2010/63/UE del Parlamento Europeo y del Consejo, de 22 de septiembre de 2010, relativa a la protección de los animales utilizados para fines científicos.

Todos los procedimientos llevados a cabo en los dos experimentos de esta Tesis doctoral siguieron los reglamentos sobre el bienestar animal que fueron aprobados por el Comité de Ética en Experimentación Animal de la Universidad de

Zaragoza, Experimento 1 (protocolo No. PI01/22); Experimento 2: ensayo 1 (protocolo No. PI31/18) y Experimento 2: ensayo 2 (protocolo No. PI35/21NE).

5.2 EXPERIMENTO UNO

Determinación de la pubertad en cerdas nulíparas: Contraste de métodos de diagnóstico.

5.2.1 Animales

Este estudio se realizó en una granja de producción porcina comercial de cerdas reproductoras ubicada en Tarragona (La Horta de Sant Joan, noreste de España). De un total de 400 cerdas nulíparas se eligieron aleatoriamente 70 cerdas (Topigs TN70, Topigs Norsvin, Madrid, España) de 240 días de edad. Las cerdas nulíparas no fueron estimuladas previamente con los verracos ni se registró previamente el estro por características de comportamiento, ni se realizó ningún tratamiento de estimulación de inducción del estro.

Las cerdas nulíparas fueron alimentadas ad *libitum con* una dieta comercial que contenía 3.200 kcal/kg EM, 15,9% PB y 1,19% de lisina digestible. Además, el agua estaba disponible *ad libitum*.

5.2.2 Diagnóstico de la pubertad

El diagnóstico de la pubertad se evaluó a ciegas para asegurar su independencia del estado de las cerdas verificadas de acuerdo con el estándar de referencia de cada método. Los diferentes métodos de diagnóstico de este ensayo fueron realizados por dos investigadores diferentes (experto y junior). La medición de las características externas del aparato reproductor de las cerdas nulíparas y la extracción de sangre para la cuantificación de la progesterona sérica (P4) se realizaron simultáneamente. Posteriormente, el mismo día, se realizó la evaluación por ecografía del aparato genital. Todas las nulíparas fueron sacrificadas al día

siguiente (16 h después) para realizar la exploración *post mortem* del aparato reproductor.

5.2.2.1 Medición de las características externas

El análisis de las características externas para el diagnóstico de la pubertad a nivel de granja se basa en datos fácilmente medibles en las cerdas nulíparas. La longitud de la vagina y el cérvix (cm) se midió utilizando un catéter calibrado (KUBUS, Madrid, España). La condición corporal se evaluó mediante puntuación visual en una escala del 1 al 5 (1 se utilizó para las cerdas extremadamente delgadas y 5 para las cerdas con sobrepeso) (Muirhead *et al.,* 1997)

Las mediciones del espesor de grasa dorsal (mm) se realizaron utilizando el método P2 (Roongsitthichai y Tummaruk, 2014). El peso vivo individual se estimó a partir del peso de la canal, utilizando la siguiente fórmula: Peso vivo peso de la canal caliente/porcentaje de perdida normal, donde el porcentaje se fijó en 70 %.

5.2.2.2 Concentración de progesterona en sangre

La extracción de muestras de sangre (10 ml) se realizó individualmente mediante venopunción yugular utilizando tubos estériles sin anticoagulante (Vacutainer Brand, Devon, Reino Unido). Las concentraciones de progesterona en suero sanguíneo (P4) se evaluaron en un laboratorio externo mediante un método analítico P4 PNT-HOR-30409, ELFA (Laboratorios CONVET S.L., Lleida, España).

5.2.2.3 Diagnostico ecográfico

Las cerdas nulíparas fueron evaluadas por ultrasonografía transcutánea en sus corrales para diagnosticar la pubertad. En una primera fase del trabajo, cada cerda fue evaluada por un técnico experto utilizando un ecógrafo de alta resolución Mylab Delta® (Esaote, Barcelona, España), ajustado a un transductor microconvexo de 8,6 MHz. En una segunda fase, todas las cerdas fueron escaneadas de forma

sucesiva e independiente utilizando un ecógrafo comercial por dos técnicos con diferentes niveles de experiencia: (experto y junior). El equipo de ultrasonidos utilizado por ambos técnicos en esta segunda fase fue un ecógrafo W3® (KUBUS, Madrid, España) ajustado a un transductor sectorial inalámbrico de 3,5 MHz.

El diagnóstico de pubertad por ultrasonido se realizó de acuerdo con el procedimiento modificado descrito por Kauffold *et al* (2019), basado en la evaluación del tamaño y posición del útero, la visualización y análisis de los ovarios. El diagnóstico de la pubertad se clasificó como "prepuberal" (PRE) y "puberal" (PUB) de acuerdo con los siguientes criterios:

- Útero: Se clasificaron como PRE a las cerdas nulíparas cuando el espacio de volumen total ocupado por el útero en su sección más ancha es 1/3 total de la sección del ultrasonido en la pantalla. La identificación de la vejiga es necesaria para una evaluación adecuada. En cambio, las nulíparas se clasificaron como PUB cuando el espacio de volumen total ocupado por el útero en su sección más ancha (la vejiga puede aparecer o no en la imagen) es 2/3 de la sección de ultrasonido total en la pantalla
- Cuernos uterinos: Los cuernos uterinos se escanearon en secciones transversales. Cuando la medida es 1 cm^2, las primerizas se clasificaron como PUB; en caso contrario, se consideraban PRE.
- Ovarios: Las cerdas PRE muestran un diámetro ovárico 2,5–3 cm con mayor tejido conectivo, visto como líneas hiperecogénicas entre los folículos < 4 mm. En la fase folicular (cerdas PUB), el tamaño folicular varía de 4 a 8,5 mm, según el momento de la ecografía en relación con la dinámica folicular.

5.2.2.4 Examen *post mortem*

El examen *post mortem proporciona* el estándar de referencia con el que se estableció la precisión de los otros métodos de diagnóstico de la pubertad en cerdas nulíparas. El estado de las cerdas PRE/PUB se evaluó sobre la base del estudio *post*

mortem del tracto genital con especial énfasis en las estructuras ováricas (Stancic *et al.,* 2011). El estado las cerdas en PUB se caracterizó por la presencia de folículos mayores de 6 mm, cuerpos *albicans* (proestro- estro), cuerpos *rubrum* (metaestro) y cuerpos lúteos (diestro). La ausencia de estas estructuras (cuerpos *albicans*, cuerpos *rubrum* y cuerpos luteos) apuntaban a un estado PRE (Falceto, 2016).

Por último, se diseccionó el tracto genital y se analizó la morfometría de cada parte por separado: se registraron las dimensiones (cm) y peso (g) de ovarios, oviductos, cuernos uterinos y cuerpo. La ausencia de cérvix y vagina en las muestras recogidas en el matadero nos impidió disponer de estos datos. También se registró el peso de la canal.

5.3 EXPERIMENTO DOS

Ensayo 1: Inseminación artificial poscervical comparada con inseminación artificial cervical en cerdas nulíparas: Evaluación de parámetros reproductivos.

Ensayo 2: Inseminación artificial poscervical única a tiempo fijo en cerdas nulíparas con buserelina. Evaluación de parámetros reproductivos.

Los dos ensayos que corresponden al experimento 2 se enfocan en la técnica de inseminación artificial poscervical y fueron realizados en granjas porcinas comerciales españolas desde el 2016 hasta el 2020. Todas las granjas se ubicaban en la C.C.A.A de Aragón, en las provincias de Zaragoza y Huesca. Un total de 1.126 cerdas nulíparas fueron utilizadas para los ensayos 1 y 2 (644 para el ensayo 1 y 482 para el ensayo 2), con una edad de 255-270 días, un peso vivo de 150 ± 5 Kg y dos celos previamente detectados.

5.3.1 Animales

Todos los animales provienen de líneas genéticas hiperprolíficas siendo diferentes en las tres granjas del primer ensayo (granja 1: Youna, AXIOM, Azay sur Indre, Francia; granja 2: Naima, Choice Genetics France, Bruz, Francia y granja 3: DanBred, DANBRED P/ S, Herlev, Dinamarca) y DanBred para para la única granja del segundo ensayo. Se seleccionadaron aleatoriamente para conformar los diferentes grupos de control y tratamiento de los dos ensayos del experimento 2.

Las cerdas nulíparas fueron alimentadas dos veces al día (3 Kg/día) con dietas comerciales formuladas para cada una de las granjas en específico según los requerimientos de cada empresa de producción porcina (3.200 Kcal EM/Kg, 14% PB y 0,7% lisina digestible). Además, para la sincronización del celo, fueron tratadas con altrenogest (REGUMATE ®, Merck & Co., Inc., Kenilworth, NJ, USA) por vía oral por 18 días. El agua fue suministrada *ad libitum.* Posterior a las IA, las cerdas nulíparas fueron alojadas en jaulas individuales (0,65 X 2 m) hasta el diagnóstico de preñez a los 24-28 días tras la inseminación.

5.3.2 Dosis seminales

En el experimento dos, se usaron 35 verracos (UPB®, Semen Cardona; CIA San Pedro, Cuarte S.A.; CIAR, España) en el primer ensayo y 49 verracos en el segundo ensayo Pietrain (Semen Costean; CIA, Costean, España) en el segundo ensayo. Los eyaculados eran recolectados una vez por semana utilizando la técnica de mano enguantada y luego filtrado para eliminar el gel o tapioca. Los verracos recibieron una dieta específica (2,6–3,0 kg, con un contenido de 3000 kcal EM/kg y 0,5 % de lisina digestiva).

La concentración espermática se evaluó mediante una cámara de conteo BRAND® patrón BLAUBRAND® Bürker (Merck & Co., Inc., Kenilworth, NJ, EE. UU); y las variables de motilidad, aglutinación y anomalías morfológicas de los espermatozoides mediante los softwares ISAS Psus® (PROISER R+D, Paterna, España)

y AndroVision® 12500/0000 (Minitube, Tiefenbach, Alemania), ISAS Psus® para el experimento 1 y AndroVision® para el experimento 2. De acuerdo con los protocolos vigentes en cada CIA, sólo se seleccionaban eyaculados que cumplían con los requisitos mínimos de motilidad y anomalías morfológicas (motilidad> 80 % y anomalías totales <20-25 %). Inmediatamente después de la evaluación microscópica, los eyaculados se procesaron como dosis heterospérmicas (2-3 eyaculados) usando un diluyente comercial a 37 °C (VITASEM®, Magapor, Ejea de los Caballeros, España). Las dosis fueron almacenadas en bolsas que contenía $1,5\times10^9$ espermatozoides en 45-60 ml para IAC o 3×10^9 espermatozoides en 90 ml para IAC. Las dosis se mantuvieron entre 15 y 18 °C durante 72 hora

5.3.3 Detección del celo

La detección de celos se realizó dos veces al día (8:00 y 13:00) en ambos ensayos, utilizando verracos maduros y observando el reflejo de inmovilidad mediante la presión de dorso de las cerdas nulíparas, así como la presencia de enrojecimiento e hinchazón de la vulva, según las pautas recomendadas por Signoret (1970). Una vez detectado el celo en una cerda, se marcó el signo "+" en su tercio posterior registrandose el día y la hora. Cuando una cerda nulípara no presentaba síntomas de celo, se consideraban en posible anestro y se retiró del experimento.

5.3.4 Inseminación artificial

Para el **ensayo 1** las cerdas nulíparas fueron inseminadas inmediatamente después del diagnóstico positivo del celo y las siguientes IA a intervalos de 12 a 24 h durante la duración del período de celo. El número medio de inseminaciones por cada cerda fue de 2,93 ± 0,29. Las cerdas del grupo IAC fueron inseminadas en presencia de un verraco. La dosis seminal (3×10^9 espermatozoides en un volumen de 90 ml) se depositó en la porción craneal del cérvix con un catéter de punta de espuma para cerdas nulíparas (Magapor, Ejea de los Caballeros, España). Para el grupo IAPC, la inseminación se realizó sin la presencia de un verraco, utilizando una

sonda IAPC específica para cerdas nulíparas (MAGAPLUS N®, Magapor, Ejea de los Caballeros, España) y como guía, el catéter con punta de espuma. Las dosis seminales para las cerdas nulíparas contenían $1,5\times10^9$ espermatozoides en un volumen de 45 ml. Se registraron las frecuencias del paso de la sonda IAPC en cada una de las inseminaciones, además de las incidencias que se presentaban durante las IA y posteriores como son el reflujo, sangrado y metritis.

Para el **ensayo 2**, las cerdas nulíparas del grupo control fueron inseminadas con la primera dosis seminal 8 horas después del inicio del celo y para la segunda dosis 12 h más tarde, durante el período de celo. Ambas inseminaciones grupo control y grupo tratamiento se realizaron mediante la técnica IAPC. En el grupo control no se indujo la ovulación, mientras ue las cerdas del grupo tratamiento recibieron g de buserelina intramuscular (Porceptal®, MSD, Salamanca, España) 120 h después de la última dosis de altrenogest. La detección de celos se realizó 30–33 horas después. Solo las cerdas nulíparas que presentaron reflejo de inmovilidad y enrojecimiento e hinchazón de la vulva fueron inseminadas en una dosis única. Tanto en el grupo control como en el tratamiento, las IAPC se realizaron sin la presencia de un verraco, utilizando una sonda IAPC específica para cerdas nulíparas (MAGAPLUS N®, Magapor, Ejea de los Caballeros, España) y, como guía, el catéter con punta de espuma (Magapor, Ejea de los Caballeros, España), y dosis seminales con $1,5 \times 10^9$ de espermatozoides en un volumen de 60 ml. Las incidencias ocurridas durante las IAPC como dificultades de paso de la sonda, reflujo o metritis se registraron.

5.3.5 Diagnóstico de gestación

La gestación se diagnosticó mediante ecografía transabdominal (Future-1®, Inserbo, España) 28 días después de las inseminaciones. La tasa de preñez se calculó como la proporción de hembras inseminadas que estaban preñadas. Después del diagnóstico positivo de gestación, se registraron retornos al celo y abortos.

5.3.6 Parto y parámetros de prolificidad

La tasa de parto se calculó como la proporción de cerdas nulíparas inseminadas que parieron. Se registraron la asistencia al parto y el número de lechones nacidos totales/camada, lechones nacidos vivos/camada, lechones nacidos muertos/camada y lechones momificados/camada. También la duración de la gestación y el intervalo destete- estro (días) se registraron para cada parto.

Para el ensayo 2, los lechones nacidos vivos se pesaron individualmente (kg) dentro de las 24 h posteriores al nacimiento y antes de las adopciones. En esta operación se utilizó una balanza calibrada (ECE 50K-2N, Kern & Sohn GmbH, Balingen, Alemania) y también, se estimó el peso de la camada (kg).

Relación de publicaciones

6

ARTICULO 1

Determination of Puberty in Gilts: Contrast of Diagnostic Methods

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Porcine Health Management

RESEARCH Open Access

Determination of puberty in gilts: contrast of diagnostic methods

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Abstract

Background: Early onset of a gilt´s puberty is needed for adequate economic performance in farms, because it indicates her reproductive performance and longevity. Therefore, an effective diagnosis is needed. Our purpose was to compare different procedures (external characteristics, blood progesterone analysis and ultrasonography diagnosis) to detect puberty in 70 gilts (Topigs TN70; 240 days old) on farm conditions. Postmortem examination was the standard reference. Multiple logistic regression analysis was used to identify which combination of independent variables (predictors) best predicts the status of gilts.

Results: Puberty (46/70 gilts; 65.71%) was characterized by the presence of follicles larger than 6 mm, *corpus albicans, corpus rubrum,* and *corpus luteum* (postmortem examination). Vaginal length, body condition, backfat, carcass weight and progesterone blood concentration were significantly higher in pubertal than prepubertal gilts ($P < 0.05$). Two types of ultrasonography equipment (DELTA and W3) were compared and performed by the same senior technician (V1). The results obtained by two technicians with different levels of experience (V1 and V2, a junior technician) using W3 were also compared. Ultrasonography provided better results than other diagnostic techniques, although the effectiveness of the ultrasonography changed with technological improvements and with increased expertise of technicians. The most accurate results were found by V1/DELTA (Nagelkerke´s $R^2 = 0.846$; Sensitivity = 0.956; Specificity = 0.958; Positive predictive value = 0.978; Negative predictive value = 0.920; Area under ROC curve = 0.957). Results using the W3 equipment could be improved when used in conjunction with vaginal length (V1; Nagelkerke´s $R^2 = 0.834$; Sensitivity = 0.933; Specificity = 0.958; Positive predictive value = 0.977; Negative predictive value = 0.885; Area under ROC curve = 0.972) or progesterone concentration (V2; Nagelkerke´s $R^2 = 0.780$; Sensitivity = 0.955; Specificity = 0.826; Positive predictive value = 0.915; Negative predictive value = 0.905; Area under ROC curve = 0.970).

Conclusions: Ultrasonography provided better results than other diagnostic techniques. The effectiveness of the ultrasonography changes with technological improvements and with increased expertise of technicians. Results using the W3 equipment could be improved when used along with vaginal length (V1) or progesterone concentration (V2). Accuracy parameters are a guide to choose puberty diagnosis, but the farms must also evaluate effect on gilts, ease and cost of administration.

Keywords: Puberty diagnosis, Gilts, Multiple logistic regression analysis, Ultrasonography, Sensibility, Specificity, Positive predictive value, Negative predictive value

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Background

Gilt productivity is important in pork production enterprises because gilts account for 20–25% of the farrowing group. In most farms, 30%-50% of the sow herd is annually replaced. Therefore, an efficient reproduction control

greatly impacts on the overall production [1, 2]. Early onset of gilt puberty is needed for adequate economic performance in commercial pig farms [3]. In fact, almost 10% of gilts are slaughtered before their first artificial insemination (AI), mainly due to reproductive problems [4]. Gilts reach puberty between 150 and 220 days-of-age [5]; therefore, accurate detection of first estrus is key to optimize the correct time of AI in their second or third estrus [6]. The onset of puberty is a reliable indicator of gilt reproductive performance and longevity [7]. The first estrus involves the efficiency of genetic potential and the physiological mechanisms that affect the sexual maturation and reproductive management in gilts [8].

Reproductive failure by delayed puberty after 7–8 months was reported as the main reason for discarding gilts [9, 10]. An increase in the age at first AI (220–300 days) was associated with 2.1% increased risk of culling due to fertility failures [8, 10]. Up to 30–40% of gilts older than 8 months did not show any sign of estrus and consequently were culled [10]. Silent estrus (ovulation without sign of estrus) occurs only in 4–5% of gilts [11]; this silent estrus may be due to an underdeveloped hypothalamic-pituitary axis without a positive feedback response by low blood estrogen concentrations [12]. However, the evidence of undetectable estrus in gilts highlights the importance of an effective reproductive management program [8]. Postmortem examination of reproductive organs showed that 60% of gilts supposed to be in anestrus had cyclical ovarian activity [8, 11, 13, 14]. Therefore, an anestrus diagnosis in gilts might be due to an inadequate estrus detection, rather than an actual absence of physiological estrus [5, 11, 14].

Currently, puberty is determined on farms by means of boar exposure and detection of signs of estrus (swollen and red vulva, interest ion boars and standing reflex in response to back pressure) [15]. Other methods proposed for detecting sexual maturity in gilts include blood progesterone analysis [16, 17], laparoscopy [18] and postmortem examination [19]. These techniques are expensive and, increase farm work and/or cause damage to animals; therefore, they are not routinely used in farms.

Certain external characteristics would be useful in detecting puberty. The study of the reproductive tract of the gilt from birth to puberty showed important changes in the weight and length of the oviducts and the uterus when puberty was reached [20]. Recently, the length of vagina-cervix has been related to length and capacity of uterine horns [21, 22]. A certain level of body condition would be needed for puberty onset in gilts [23]; also, fat mass was associated with puberty in female mammals [24]. Growth patterns, easily measurable in farm, would be predictive of puberty onset in gilts [25]; therefore, the length of vagina-cervix, body condition and backfat would be valuable in detecting puberty. In sows, ultrasonography has proved to be useful in detecting pregnancy, estimating time of ovulation and determining ovarian pathology [26]. Also, ultrasonography allows visualization of gilt uterus and ovaries and is considered highly sensitive for puberty diagnosis [27–30].

The purpose of this study was to compare different procedures (external characteristics, blood progesterone analysis and ultrasonography diagnosis) in terms of their ability to detect puberty in gilts on farm conditions. Moreover, two types of ultrasonography equipment were compared when used by the same technician. Also, the results from two technicians with different levels of experience were compared when using the same equipment. Postmortem examination was the standard reference.

Materials and methods

Animals

This study was performed in accordance with the European Directive for pig protection [31] and the Spanish legislation for animal protection in experimentation and other scientific purposes, including teaching [32]. Expert veterinarians were in charge of caring and handling the animals. The Ethical Committee for Animal Experiments, University of Zaragoza, Spain approved this study (reference number: PI01/22).

This study was conducted according to the Spanish standard commercial swine production on a breed farm located near Tarragona (La Horta de Sant Joan, Southeastern Spain). Out of a total of 400 gilts housed in 40 pens (10 gilts/pen, pen size: 2×5 m), 70 gilts (Topigs TN70, Topigs Norsvin, Madrid, Spain) were randomly chosen to use in the study; these gilts were 240 days old. Gilts were not previously exposed to boars nor was estrus previously checked by behavioral characteristics. No estrus-stimulating treatment was performed. Several reasons explained for these decisions in the study design. We intended to contrast several puberty diagnostic methods as blind tests. Moreover, our previous experience showed that at 240 days of age the gilts could be pubertal or not, and the detection of their pubertal status was the basis for this contrast of methods. Gilts were fed ad libitum with a commercial finishing diet containing 3200 kcal/kg metabolizable energy (ME), 15.9% crude protein (CP), and 1.19% digestible lysine. Also, water was available ad libitum.

Puberty diagnosis

The results from several diagnostic methods were blindly assessed to assure their independence from verified gilts status according to the reference standard. Each

diagnostic method of this blind study was performed by different researchers. The measurement of the external characteristics and the extraction of blood for the quantification of serum progesterone (P4) were carried out simultaneously. Later on the same day, the ultrasonography was carried out. All gilts were slaughtered the day after the scan (16 h afterwards).

External characteristics

Farm diagnosis of puberty is based on several external characteristics, easily measurable on gilts in farm condition. Vaginal length (cm) was measured using a calibrated catheter (KUBUS, Madrid, Spain). Body condition was evaluated by visual scoring on a scale ranging from 1 to 5: 1 was used for extremely thin sows and 5 for extremely fat ones [33]. Backfat measurements (mm) were performed using the P2 method [9].

Individual live weight was not measured but it was estimated from carcass weight, by using the following formula**:** Live weight = hot carcass weight/typical dressing percentage, where typical dressing percentage was set at 70% [34].

Progesterone concentration

Blood sample collection (10 ml) was individually performed by jugular venipuncture using sterile tubes without additives (Vacutainer Brand, Devon, UK).

Blood serum progesterone concentrations (P4) were assessed in an external laboratory by a P4 analytical method PNT-HOR-30409, ELFA (Laboratorios CONVET S.L., Lleida, Spain).

Ultrasonography

The gilts were submitted to transcutaneous ultrasonography in their pens; both ultrasonography equipment are portable. In a first phase of the study, every gilt was studied by a senior, expert technician (V1) using the high-resolution ultrasound Mylab Delta® (Esaote, Barcelona, Spain), adjusted to 8.6 MHz microconvex transducer (henceforth Delta). In a second phase, all gilts were successively and independently scanned using the same equipment by two technicians with different levels of expertise: V1 (senior) and V2 (junior). The ultrasound equipment used by both technicians in this second phase was a commercial ultrasound W3® (KUBUS, Madrid, Spain) adjusted to a 3.5 MHz wireless sectorial transducer (henceforth W3). In total, three scans were conducted on every gilt.

Ultrasound puberty diagnosis was performed according to a modified procedure described by Kauffold et al. [30], based on the evaluation of uterus size and position and the visualization and analysis of the ovary. The transducer was placed horizontally on the right or left ventrolateral abdominal wall just dorsal to the last pair of teats.

Sexual maturity was expressed as "prepubertal" (PRE) and "pubertal" (PUB) according to the following criteria, that must be fulfilled simultaneously:

(1) *Uterus*: Gilts were classified as PRE when during the scan of the bladder, the total volume space occupied by the uterus in its widest section is $\leq 1/3$ total of the ultrasound section on the screen. Identification of the bladder is necessary for a proper assessment. Instead, gilts were classified as PUB when the total volume space occupied by the uterus in its widest section (the bladder may or may not appear in the image) is $\geq 2/3$ total ultrasound section on the screen.
(2) *Uterine horns*: The uterine horns were scanned in cross-sections. When the measured digital strip was $\geq 1 cm^2$, gilts were classified as PUB; otherwise, they were considered as PRE.
(3) *Ovary*: The PRE gilts shows a major ovarian diameter $\leq 2.5–3$ cm with obvious connective tissue, seen as hyperechoic lines holding < 4 mm follicles. In follicular phase (PUB gilts), follicular size varies from 4 to 8.5 mm, based on timing of ultrasonography relative to ovulation. In proestrus, follicular size varies 5–5.5 mm and in estrus, follicular size exceed 5.5 mm. In metaestrus, corpus rubrum occurs and in diestrus, corpus luteum (5–10 mm) appears.

The time required for every ultrasound procedure/technician was also recorded.

Postmortem examination

The postmortem examination provides the reference standard to which the accuracy of the other diagnostic methods was stablished. Gilt status (PRE/PUB) was assessed on the basis of postmortem study of the genital tract with special emphasis on the ovarian structures [11]. PUB status was characterized by presence of follicles larger than 6 mm, *corpus albicans* (PUB in proestrus-estrus), *corpus rubrum* (PUB in metaestrus) and *corpus luteum* (PUB in diestrus). The absence of these structures (*corpus albicans, corpus rubrum* and *corpus luteum*) pointed to PRE status.

Also, the genital tract was dissected and the morphometry of each part was separately analyzed: dimensions (cm) and weight (g) of ovaries, oviducts, uterine horns and body

were recorded. The absence of cervix and vagina in postmortem collected at the slaughterhouse prevents us from having these data**.** Carcass weight was also recorded.

Statistical analysis

Statistical analyses were performed by using IBM SPSS version 26 software (SPSS, Chicago, IL, USA). Means and standard deviation (SD) summarize the quantitative variables (morphometric data, weight, backfat, body condition, P4, and times for ultrasound procedures) and counts of *corpus rubrum*, luteum and albicans.

Follicles sizes were grouped in semi-open intervals starting from the first category (≤ 1 mm). Interval semi-open on the left (a, b] is the set of all real numbers greater than "a" and less than or equal to "b". In this way, eight categories for follicles size were created. For every individual, percentage of each category was calculated on total follicles number. Means and SD were also estimated for each category.

One–way ANOVA (analysis of variance) was applied to comparisons between PRE and PUB groups for carcass weight and backfat. Comparisons between groups for morphometric variants were carried out by ANCOVA (analysis of covariance) where carcass weight was included as covariate. A non-parametric test (Mann–Whitney U test) compared distribution of follicles size intervals, body condition and P4 between groups. Friedman test (non-parametric) was used to compare needed time among ultrasound procedures.

Cohen´s κ was run to determine if there was agreement in the ultrasound test results from the same individuals in two situations: (1) two types of equipment (Delta and W3) used by one technician (V1) and (2) the same equipment (W3) used by two technicians (V1 and V2). As usual, the greater the value of κ, the greater the strength of the agreement (< 0.20: poor; 0.21–0.40: weak; 0.41–0.60: moderate; 0.61–0.80: good; 0.81–1.00; very good) [35].

Multiple logistic regression analysis was used to identify which combination of independent variables (predictors) best predicts the status of gilts (dependent variable: PRE or PUB). A stepwise procedure (Method: forward) was applied; independent variables moved in or out of the model at any step of the process, on the basis of the Wald test, which determined statistical significance for each of the independent variables: the significance levels to enter and to be removed were $P \leq 0.05$ and $P \geq 0.10$, respectively [36]. When ultrasound procedure results were considered as independent variable, the reference level was PRE (coded as 0), to which the other one (PUB, coded as 1) will be compared. Model fit was assessed by chi squared test (omnibus test of model coefficients) that provides the overall statistical significance of the model. Nagelkerke R^2 estimates how much variation in the dependent variable can be explained by the model. A cut–off point of 0.5 was used and the gilt status will be classified as PUB only if its predicted probability was ≥ 0.5. The ability of models to discriminate between PRE or PUB individuals was assessed by estimating sensitivity (true positive rate), specificity (true negative rate), positive and negative predictive values (proportions of positive and negative results in diagnostic tests that are true positive and true negative results, respectively) [37]. The area under the Receiver Operating Characteristics (ROC) curve estimates an overall measure of discrimination [38].

P values < 0.05 were considered as statistically significant.

Results

Puberty was assessed in 46/70 (65.71%) studied gilts by means of postmortem examination (reference standard). Distribution of follicle size intervals are shown in Fig. 1.

Significant differences ($P < 0.05$) were found among PRE and PUB groups for percentage of follicles larger than 6 mm. Number of *corpus rubrum*, *corpus luteum* and *corpus albicans* in PUB gilts are showed in Table 1.

The carcass was significantly heavier ($P < 0.001$) in the PUB group (105.12 ± 14.666 kg) than in the PRE group (91.17 ± 15.189 kg). Table 2 shows dimensions and weight of genitalia from postmortem examination. Significant differences were found in every case, with higher dimensions and weight in the PUB group ($P < 0.001$).

Results for the parameters measured on the farm (vaginal length, body condition, backfat, and estimated live weight) and P4 are shown in Table 3. This table also shows data for progesterone concentrations. Pubertal gilts always showed higher values ($P < 0.05$).

Figures 2, 3, 4 and 5 show ultrasonography images from V1/Delta. It is noteworthy that explaining an echography by an only image is very difficult. Usually, one frame is selected, where the structure under study is represented.

In Fig. 5, an ovary is clearly visible in the center of the image. Around it, there is a venous plexus than could be confused with follicles in a still image like this; a moving image would clearly capture the difference between these structures.

Figures 6 and 7 show ultrasonography images from V2/W3.

Results from ultrasonography tests are shown in Table 4. One gilt could not be examined by the V2 technician using the W3 equipment.

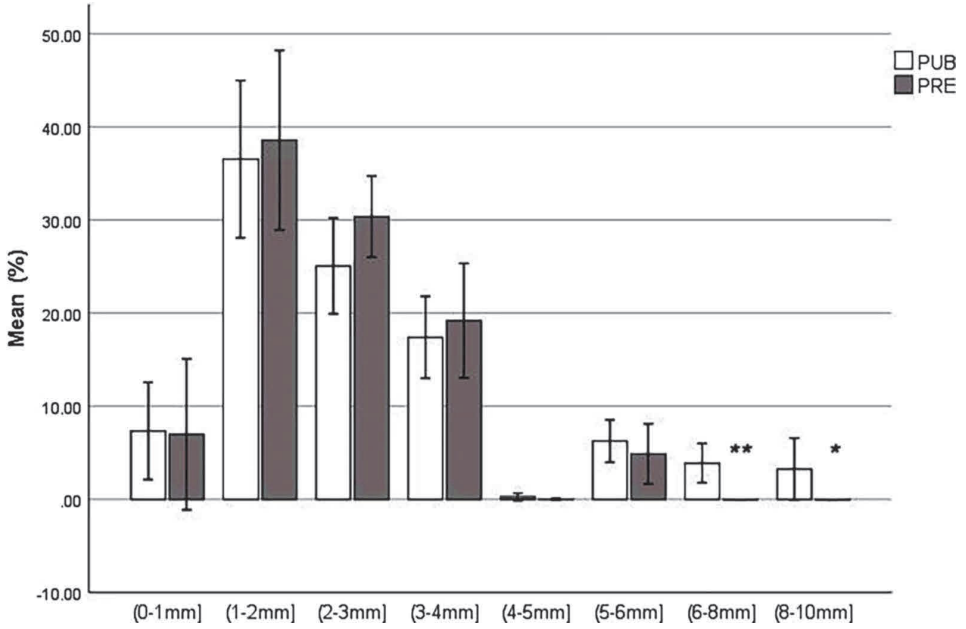

Fig. 1 Distribution of follicles size intervals (%) from postmortem examination. Error bars: 95% CI;*: $P < 0.05$; **$P < 0.001$

Table 1 Number of corpus rubrum, luteum and albicans in PUB gilts (Mean ± SD)

Ovary side and *corpus*	Mean ± SD
Left ovary corpus *rubrum*	3.15 ± 4.269
Right ovary *corpus rubrum*	3.57 ± 4.778
Left ovary *corpus luteum*	4.61 ± 5.931
Right ovary *corpus luteum*	3.98 ± 4.842
Left ovary *corpus albicans*	9.09 ± 6.390
Right ovary *corpus albicans*	8.20 ± 5.837

Cohen´s κ for V1 using the two types of equipment (Delta and W3) was 0.848 ($P < 0.001$), indicating very good concordance. Good concordance (0.668) was found for V1 and V2 using the W3 equipment (Cohen´s κ = 0.668; $P < 0.001$).

The time required for V1/W3 (12.57 ± 11.143 min) was significantly shorter than for both V1/Delta (17.74 ± 10.754 min; $P = 0.002$) and V2/W3 (20.20 ± 15.694 min; $P = 0.004$). No significant differences were found between V1/ Delta and V2/W3 ($P = 0.833$).

Logistic regression models, successively adjusted, are shown in Table 5. All of them were statistically significant ($P < 0.001$), demonstrating a good model fit. For model I, vaginal length, body condition, backfat and live weight (estimated) were used as independent variables. Model II added P4 as independent variable. Models III, IV and V added results from ultrasonography (one technician/equipment in turn) as independent variables. Finally, models VI and VII included only results from V1/W3 and V2/W3, respectively. Table 5 shows which independent variables were chosen as better predictors in each model. As can be seen, in models II and V two independent variables with P value > 0.05 were kept in the best model fit (backfat and P4, respectively); in both models, inclusion of these variables improved fit and explained the percentage of prediction variation.

Initially, model I considered morphological characteristics easily measurable in farm (external characteristics diagnosis) as independent variables: vaginal length and backfat were chosen as best pubertal predictors. When progesterone concentration was considered together with external characteristics, only backfat and P4 were chosen (model II). Once results from V1/Delta were considered, only this variable was chosen (model III). However, when results from V1/W3 and V2/W3 were considered, best fit models also includes vaginal length and P4, respectively (models IV and V). Models including only results from V1/W3 and V2/W3 also were significant (models VI and VII).

Table 2 Morphometry of genital tract from postmortem examination (Mean ± SD)

Trait	PRE (n = 24)	PUB (n = 46)	*P* Value
Right ovary thickness (cm)	1.09 ± 0.230	1.59 ± 0.401	< 0.001
Left ovary thickness (cm)	1.11 ± 0.238	1.57 ± 0.392	< 0.001
Right ovary height (cm)	2.05 ± 0.209	2.64 ± 0.466	< 0.001
Left ovary height (cm)	2.15 ± 0.257	2.75 ± 0.540	< 0.001
Right ovary length (cm)	2.95 ± 0.365	3.82 ± 0.511	< 0.001
Left ovary length (cm)	2.99 ± 0.322	3.94 ± 0.697	< 0.001
Right ovary weight (g)	3.21 ± 0.815	5.94 ± 2.262	< 0.001
Left ovary weight (g)	3.53 ± 0.923	6.65 ± 2.868	< 0.001
Right oviduct length (cm)	20.22 ± 2.280	28.24 ± 3.787	< 0.001
Left oviduct length (cm)	21.23 ± 3.159	30.11 ± 3.707	< 0.001
Right oviduct weight (g)	1.01 ± 0.302	2.07 ± 0.536	< 0.001
Left oviduct weight (g)	1.05 ± 0.308	2.11 ± 0.508	< 0.001
Right uterine horn diameter (cm)	1.72 ± 0.431	2.76 ± 0.512	< 0.001
Left uterine horn diameter (cm)	1.72 ± 0.431	2.80 ± 0.572	< 0.001
Right uterine horn length (cm)	64.73 ± 11.536	128.71 ± 30.230	< 0.001
Left uterine horn length (cm)	68.33 ± 12.269	131.91 ± 33.030	< 0.001
Right uterine horn weight (g)	42.61 ± 22.293	251.64 ± 90.360	< 0.001
Left uterine horn weight (g)	43.38 ± 22.694	245.25 ± 91.348	< 0.001
Right uterine horn thickness (cm)	0.13 ± 0.086	0.42 ± 0.139	< 0.001
Left uterine horn thickness (cm)	0.13 ± 0.086	0.42 ± 0.139	< 0.001
Uterine body length (cm)	2.59 ± 0.757	3.89 ± 0.843	< 0.001
Uterine body weight (g)	3.06 ± 1.410	11.38 ± 4.268	< 0.001

Table 3 Farm parameters and progesterone concentration (Mean ± SD)

Traits	PRE (n = 24)	PUB (n = 46)	*P* Value
Vaginal length (cm)	21.75 ± 3.848	27.35 ± 5.435	0.003
Body condition (1–5 pts)	3.13 ± 0.338	3.43 ± 0.501	< 0.001
Backfat (mm)	6.46 ± 1.931	9.50 ± 2.469	< 0.001
Live weight (kg)	130.24 ± 21.693	150.16 ± 20.970	< 0.001
P4 ($ngmL^{-1}$)	0.84 ± 0.414	29.10 ± 27.941	< 0.001

Table 6 shows the accuracy parameters of the seven logistic regression models (Nagelkerke's R^2, Sensitivity, Specificity, Positive predictive value, Negative predictive value, Area under the ROC curve).

Models I and VII, respectively based on external characteristics and V2/W3, showed lowest accuracy values. External characteristics based diagnosis improved when progesterone concentration was considered with backfat (model II). Also, V2/W3 improved when considered with P4 for pubertal diagnoses (model V). Results from V1/W3 became more accurate when vaginal length was also considered (model IV). Finally, best accuracy was obtained only from V1/Delta (model III); even though area under ROC curve was lower for model III than for models IV and V, their 95% IC widely overlapped.

Discussion

The onset of puberty is a complex physiological process where endocrine and physical factors are associated to achieve sexual maturation. The age at puberty onset is in part controlled by individual genetics (moderately heritable, r = 0.38) and can show individual variability [39, 40].

Due to the failure of puberty diagnosis, about 30–60% of gilts are culled, causing a severe economic impact in modern commercial farms [41, 42]. This percentage of culled gilts could be reduced by an improved techniques and effectiveness of estrus detection.

In the present study, postmortem examination was used as a reference standard to represents the actual situation or as close to it as current measures allow. In PRE gilts, the ovaries are characterized as honeycomb (1-3 mm follicles), grape (up to 6 mm) or intermediate type [13, 43]. In the PRE gilts, follicles seem to be recruited in waves, but only grow to 6 mm in size before undergoing atresia [12]. Therefore, distribution of follicle size was similar in both groups up to 6 mm; follicles larger that 6 mm were only present in PUB gilts. The number of *corpus luteum* and *corpus albicans* in both ovaries in PUB gilts are compatible with a normal first cycle estrus [13].

PRE and PUB gilts clearly differed in the development of different sections of the genital tract, as previously described for length of uterine [44, 45] and uterine sections [25, 46]. Furthermore, increased follicular development was accompanied by increased size of all uterus sections [25, 46].

Both internal (breed, body weight, backfat) and management (nutrition, boar contact, surroundings) factors control puberty in gilts, mediated by the endocrine-reproductive axis [28]. Gilts with a high growth rate attained puberty earlier than those with low growth rate [47, 48]. Body weight and backfat have an impact on gilt reproduction. Releasing of gonadotropins and maturation of ovarian follicles depend on body weight and fat [49], growth rate and age [50, 51]. The particular effects of these factors are difficult to ascertain, but slow

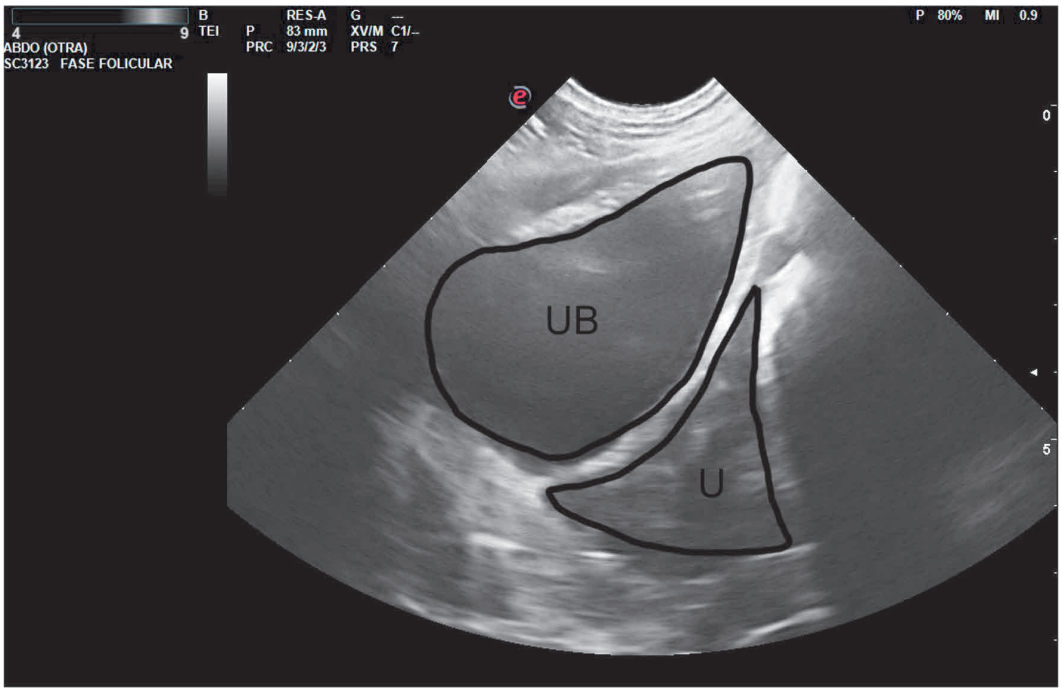

Fig. 2 Prepubertal gilt (V1/Delta). The urine bladder (UB) appears as an anechoic structure in the center of the image, just below the small uterus (U), well delimited by the intestinal loops

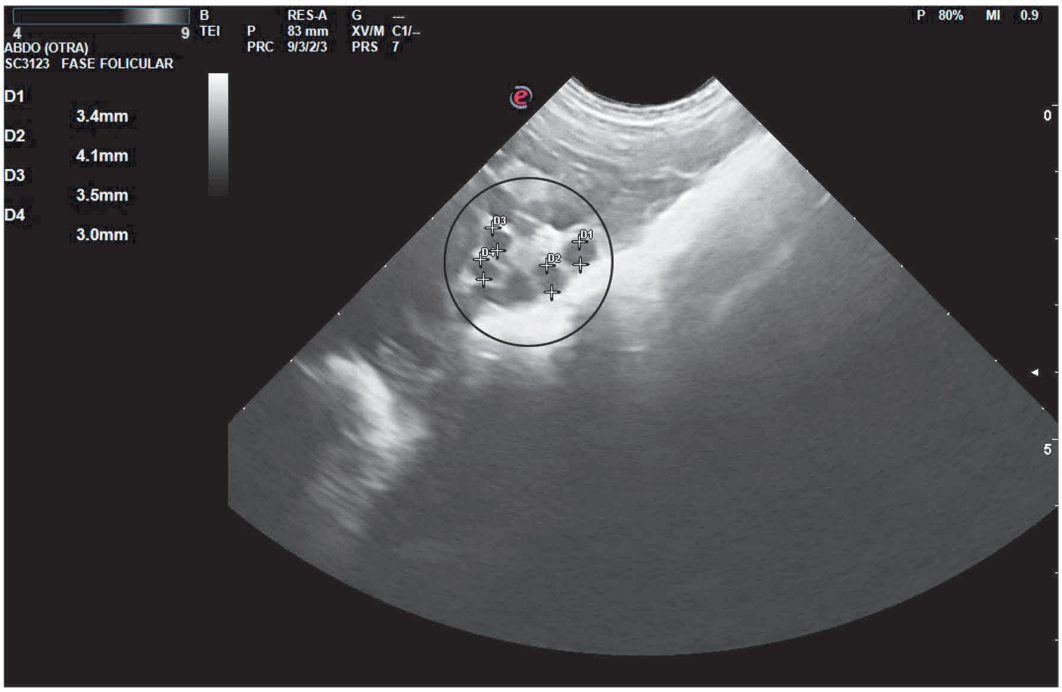

Fig. 3 Prepubertal gilt (V1/Delta). Small ovary (2.7 cm) and follicles (2–4 mm; inside the circle)

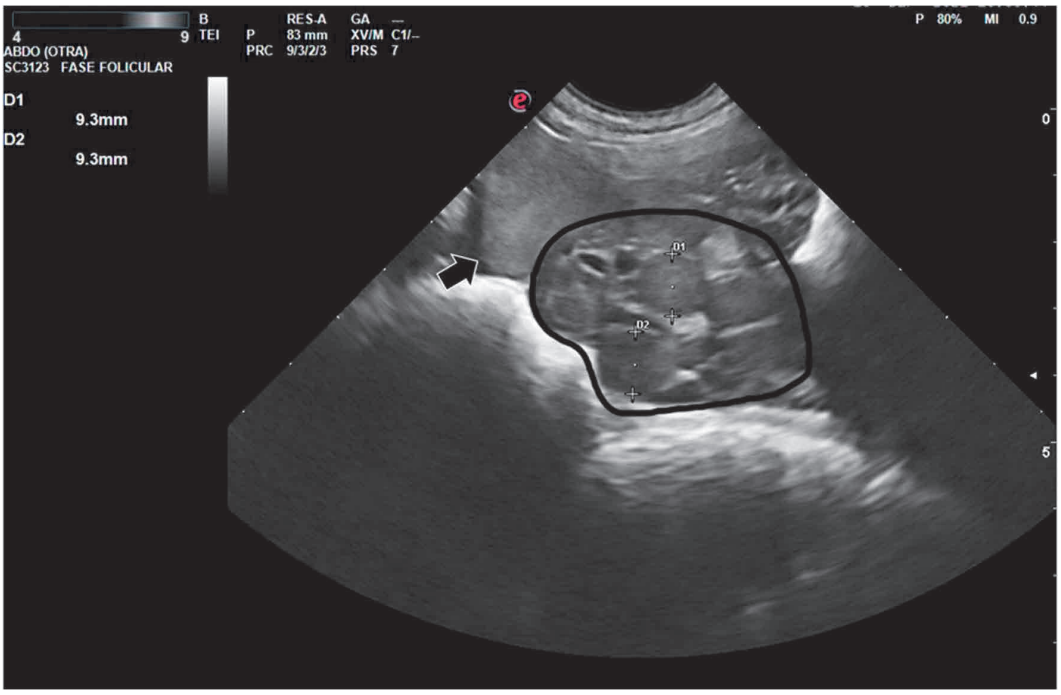

Fig. 4 Pubertal gilt (V1/Delta). Inside the circle: ovary with corpora lutea. Five corpora lutea are clearly visible; one more corpus luteus would be occult. Two of them were measured (approximately 9 mm each diestrum middle phase). The intestinal loops can be seen under the ovary. The arrow signals the section of a uterine horn (diameter: 2 cm)

growing gilts are lighter and show both thinner backfat at selection and delayed puberty, being more likely to be culled [52].

Backfat has been related with puberty onset [53]. Gilts with high backfat (17.8 mm), fed ad libitum, reached puberty at 198 days of age, whereas those with low backfat (14.7 mm), restricted to 80% feed, attained puberty at 203 days of age [54]. Heritability for age at puberty ($h^2 = 0.3$) has been reported as slightly higher than for other reproductive traits [54]; therefore, the selection of replacement gilts on the basis of backfat could contribute to excellent reproductive performance of the herd. Tummaruk et al. [48] showed that gilts had their first estrus at 195 days of age with 106 kg body weight and 11 mm backfat, on average, but marked differences in the weight and backfat were found.

Under farm condition, objective assessment of body condition is not easy. Assessment of body condition is based on visual examination of fatness, scores ranging from 1 to 5. Given that this evaluation relies on personal scoring skills, it is regarded as an imprecise and subjective method [9]. Also, live weight was not directly measured, but estimated from carcass weight, as mentioned in Material and methods. The exclusion of these variables from the predictive equation (model I) could be explained by their low accuracy.

On the other hand, the relationship between age, body weight, body composition, and puberty onset is controversial. Dietary treatments do not seem to affect pubertal age [55]. As reviewed by Rauw et al. [56], gilts with a greater lean percentage had a delayed onset of puberty, and negative genetic correlations have been reported between growth rate and estrus signs at puberty [54, 57]. Dietary conditions and exposure to mature boards was more related with puberty onset in gilts than minimum threshold amount of body tissues or a specific rate of body reserves [58]. These facts would explain for the lower accuracy of model I, at least in the described farm conditions.

Eliasson [59] highlighted the importance of progesterone analysis in puberty diagnosis. Progesterone concentration only increases after puberty, following the

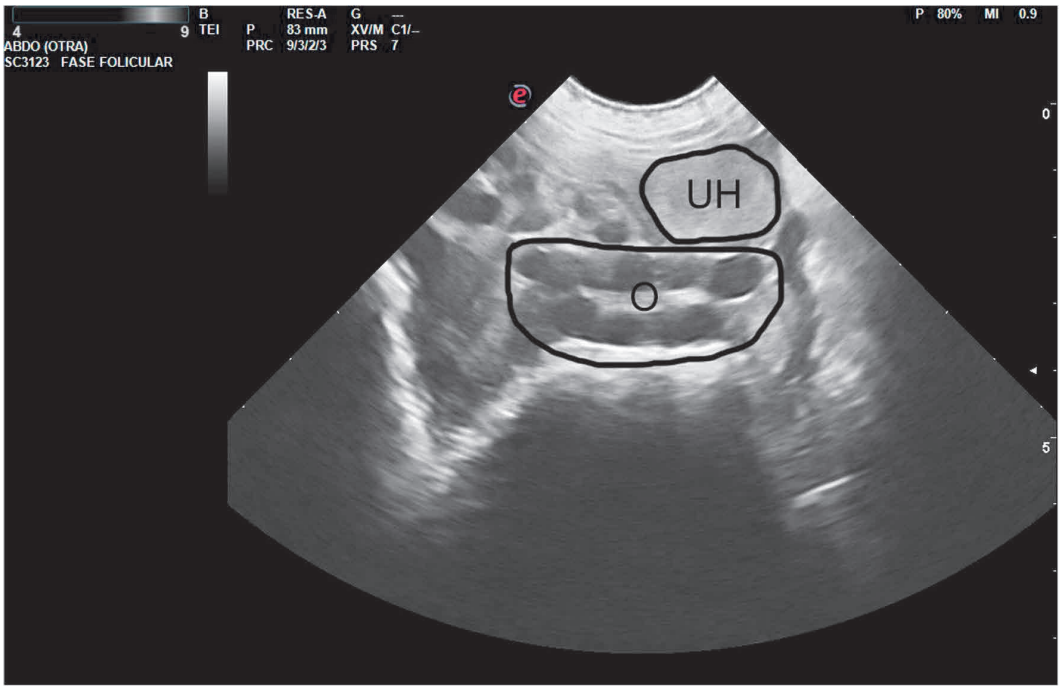

Fig. 5 Pubertal gilt (follicular phase, V1/ Delta). The ovary (O) with preovulatory follicles can be seen in the center of the image. Above the ovary, the section of a uterine horn (UH) can be seen (diameter: 2 cm). Under the ovary, the image shows the intestinal loops

formation of the first *corpus luteum* [27, 28]. Therefore, gilts showing progesterone concentration > 2ngmL-1 will be considered as pubertal gilts but below this value they will be classified as prepubertal ones [29, 60]. We found than P4 greatly differed between pubertal and prepubertal gilts, with higher values for pubertal ones; P4 is a good puberty marker. As stated before (see "Statistical analysis"), P4 was proposed as independent variable only for models II- V. The stepwise procedure used for fitting logistic regression models chose independent variables for remaining in the model on the basis of their statistical significance, taking into account the effects of the other independent variables included in the model. Hence, P4 was retained only in models II and V, because including this variable improved models fit. Therefore, better accuracy parameters were obtained when P4 was added to models where less powerful independent variables were previously included (backfat and V2/W3, respectively). However, when V1/Delta and V1/W3 were retained in models III and IV respectively, power for detecting puberty was so high than no benefit was accomplished by including P4 in these models.

Ultrasonography has been recommended as a reliable and less laborious method for puberty in gilts for both research and farm, reporting an accuracy of 95–100% (percent of gilts correctly classified by ultrasonography as PRE or PUB) when both uterus and ovaries are examined [29, 61]. Ultrasonography can detect ovaries and their structures; even small follicles can be due to its anechoic appearance [62]. In the transition from PRE to PUB status, the uterus grows strongly and the uterine horn diameters increase [61]. Therefore, while the prepubertal uterus is a small structure needing more time for visualization, the pubertal uterus can be seen quickly [29]; even subjective determination of pubertal status in gilts, as described above, would be reliable for puberty diagnosis.

The present work compares different procedures based on their ability to detect puberty in gilts on farm conditions, assessed by the accuracy parameters (Nagelkerke's R^2, Sensitivity, Specificity, Positive predictive value, Negative predictive value, Area under the ROC curve). These parameters can be used as guidance for choosing the diagnosis procedure and this is an important result of this work. As shown, the most accurate results were

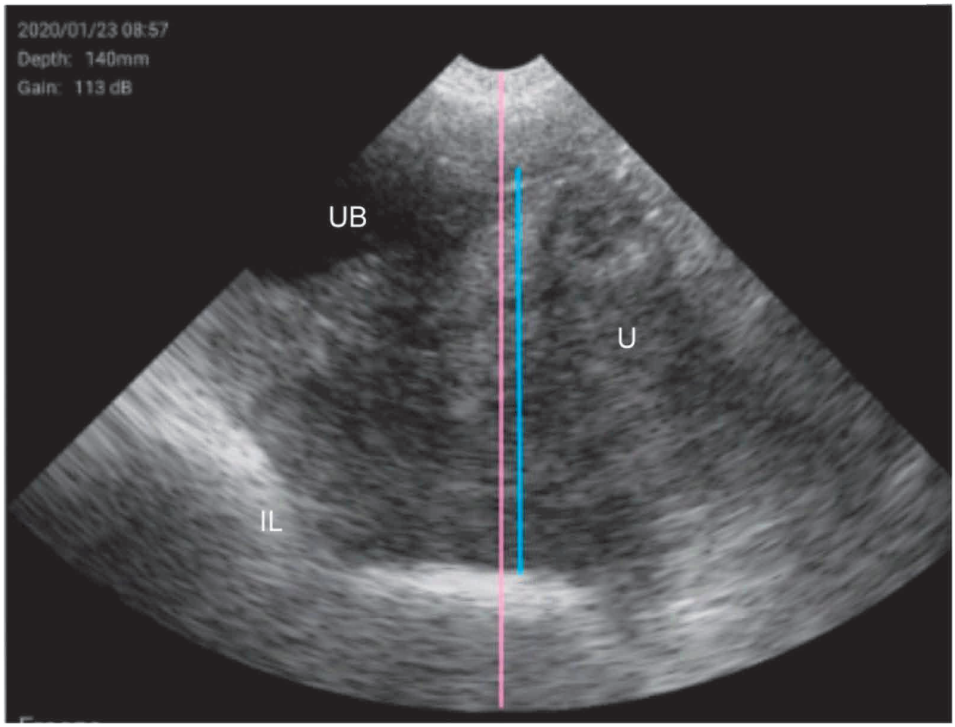

Fig. 6 Pubertal gilt (V2/W3). Urine bladder (UB), intestinal loop (IL) and uterus (U) appear as well distinguishable structures. UB shows a completely anechoic structure typical of liquids. IL is characterized by the gas hyperechogenicity. U is a central, homogeneous and echogenic structure situated below and in front of UB; it occupies almost the entire screen. The U height (in blue) is greater than two-thirds of the total height of the image (in pink), which highlights the large volume of the uterus, characteristic of a pubertal gilt

obtained by V1/Delta (model III), followed by V1/W3 when used in conjunction with vaginal length (model IV) and V2 /W3 with progesterone concentration (model V). However, in farm practice, additional factors must be considered in the choice of puberty diagnosis procedure.

We considered two ultrasound equipments with different characteristics. Designed for pregnancy diagnosis at every gestational age, they do not require a specific installation and adapt to any situation. Ultrasound equipment W3® is a commercial device with an affordable cost for all companies in the sector, widely distributed worldwide because it is totally portable and easy to use. The high-resolution ultrasound Mylab Delta® is professional equipment, robust and portable, that needs a qualified technician and has a high cost. Time required for puberty diagnoses depends on both equipment and technician experience, mean values being lower for W3 and experienced technicians.

Implementing ultrasonography diagnosis can be a challenge on some farms due to the necessary investment in equipment purchase and/or basic technician training. Even in best ecographical images, some ambiguity remain for the untrained eye; this is one of the more frequent criticism about these techniques for be used in farms. Therefore, puberty diagnosis based on backfat and progesterone concentration (model II) would be sufficient enough in view of its accuracy values, avoiding new investments.

Conclusions

Ultrasonography provided better results than other diagnostic techniques, although V2 obtained the worst results. These results highlight the need for experienced technicians. The most accurate results were obtained by V1/Delta: the effectiveness of the ultrasonography changes with technological improvements and with

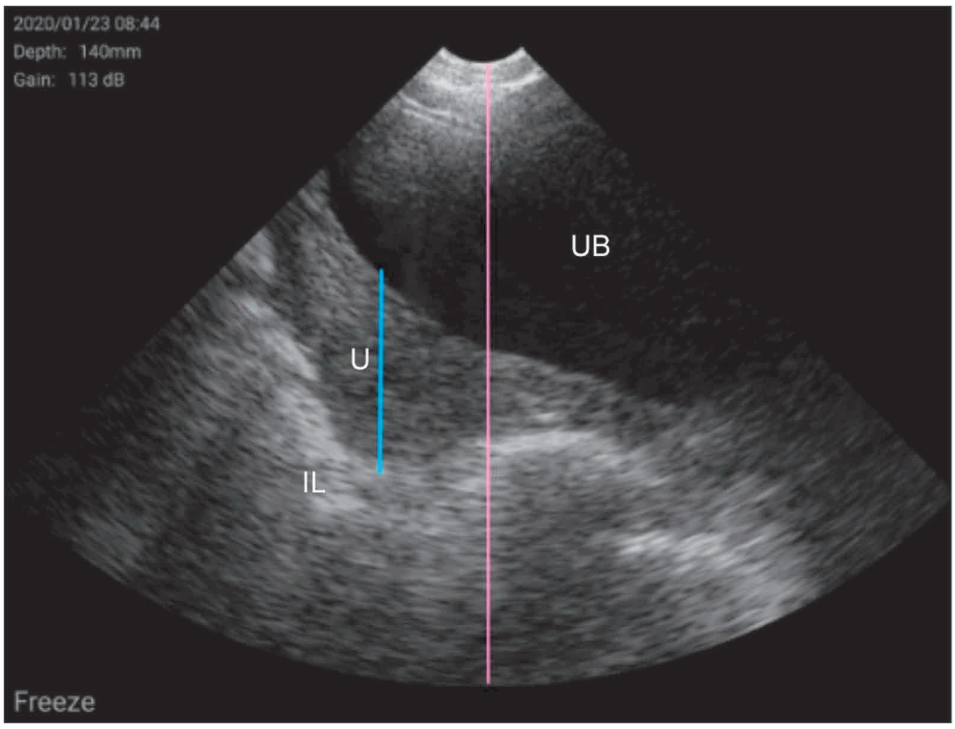

Fig. 7 Prepubertal gilt (V2/W3). Urine bladder (UB), intestinal loop (IL) and uterus (U).UB (completely anechoic structure) shows a great volume of urine. The gas hyperechogenicity allows distinguishing IL (white line). U appears as a homogeneous and echogenic structure situated between UB and IL. The U height (in blue) is smaller than two-thirds of the total height of the image (in pink); this means that U is not yet fully developed because uterine inactivity, characteristic of a prepubertal gilt

Table 4 Characteristics of ultrasonography tests

Technician/Equipment		Reference standard		Total
		PRE	PUB	
V1/Delta	PRE	23	2	25
	PUB	1	44	45
	Total	24	46	70
V1/W3	PRE	23	5	28
	PUB	1	41	42
	Total	24	46	70
V2/W3	PRE	19	9	28
	PUB	4	37	41
	Total	23	46	69

increased expertise of technicians. Results from W3 procedure could be improved when used in conjunction with vaginal length (V1) or progesterone concentration (V2).

Accuracy parameters can be used as guidance for choosing the diagnosis procedure but procedures can also be compared based on ease of administration, cost of administration, and effect on patients (invasiveness, discomfort, convenience). In this sense, ultrasonography equipment is usually present in farms due its use in pregnancy diagnosis and in these cases, ultrasonography is cheaper than progesterone concentration analysis. Also, it causes less discomfort than vaginal length measurement and takes less time when used by an experienced technician. However, ultrasonography diagnosis needs investment in equipment purchase and/or basic technician training. As shown, puberty diagnosis based on backfat and progesterone concentration could be a good alternative, in view of its accuracy values.

Table 5 Models of multiple logistic regressions

Model	Variable	Coefficient (β)	Standard error	Wald χ^2	*P* Value	Odds ratio	95% CI		Variables not in the equation
							Lower	Upper	
I	Intercept	−8.666	2.365						Body condition, live weight (estimated)
	Vaginal length (cm)	0.22	0.083	7.096	0.008	1.247	1.060	1.466	
	Backfat (mm)	0.511	0.155	10.832	0.001	1.666	1.229	2.258	
II	Intercept	−5.036	1.525						Body condition, live weight (estimated), vaginal length
	Backfat (mm)	0.338	0.173	3.816	0.051	1.402	0.999	1.968	
	P4 (ng/ml)	1.907	0.955	3.984	0.046	6.731	1.035	43.768	
III	Intercept	3.761	1.012						Body condition, live weight (estimated) vaginal length, backfat, P4
	V1 /DELTA	−6.204	1.252	24.563	< 0.001	0.002	0.000	0.024	
IV	Intercept	−7.645	4.243						Body condition, live weight (estimated) backfat, P4
	Vaginal length (cm)	0.544	0.234	5.420	0.020	1.723	1.090	2.725	
	V1/W3	−7.150	2.224	10.334	0.001	0.001	0.000	0.061	
V	Intercept	−0.341	1.396						Body condition, live weight (estimated), vaginal length, backfat
	P4 (ng/ml)	1.279	1.115	1.314	0.252	3.592	0.403	31.969	
	V2/W3	−2.993	0.991	9.127	0.003	0.050	0.007	0.349	
VI	Intercept	3.714	1.012						
	V1/W3	−5.240	1.126	21.653	< 0.001	0.005	0.001	0.048	
VII	Intercept	2.225	0.526						
	V2 /W3	−2.972	0.664	20.037	< 0.001	0.051	0.014	0.188	

Table 6 Accuracy parameters of the logistic regression models

Model	Parameter					
	Nagelkerke's R^2	Sensitivity (95%IC)	Specificity (95%IC)	Positive predictive value (95%IC)	Negative predictive value (95%IC)	Area under the ROC curve (95%IC)
I	0.520	0.844 (0.738; 0.950)	0.792 (0.629; 0.954)	0.883 (0.787; 0.979)	0.731 (0.561; 0.901)	0.882 (0.806; 0.959)
II	0.722	0.867 (0.767; 0.966)	0.875 (0.743; 1.000)	0.928 (0.850; 1.000)	0.778 (0.621; 0.935)	0.943 (0.890; 0.996)
III	0.846	0.956 (0.897; 1.000)	0.958 (0.878; 1.000)	0.978 (0.935; 1.000)	0.920 (0.814; 1.000)	0.957 (0.900; 1.000)
IV	0.834	0.933 (0.860; 1.000)	0.958 (0.878; 1.000)	0.977 (0.932; 1.000)	0.885 (0.764; 1.000)	0.972 (0.937; 1.000)
V	0.780	0.955 (0.895; 1.000)	0.826 (0.671; 0.981)	0.915 (0.835; 0.995)	0.905 (0.780; 1.000)	0.970 (0.933; 1.000)
VI	0.746	0.891 (0.801; 0.981)	0.958 (0.878; 1.000)	0.976 (0.929; 1.000)	0.821 (0.680; 0.962)	0.925 (0.854; 0.996)
VII	0.442	0.804 (0.690; 0.919)	0.826 (0.671; 0.981)	0.902 (0.812; 0.992)	0.678 (0.506; 0.850)	0.815 (0.703; 0.897)

Abbreviations

P4: Progesterone concentration; V1: Expert technician; V2: Junior technician; Delta: Ultrasound Mylab Delta; W3: Ultrasound W3; PRE: Prepubertal; PUB: Pubertal; SD: Standard deviation; ANOVA: Analysis of variance; ANCOVA: Analysis of covariance; SE: Standard error; CI: Confidents intervals.

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Author contributions
External characteristics were studied by AS-U, SM and ML. Progesterone analyses were supervised by OM. Ultrasonography diagnosis were carried out by AV (experienced technician, V1) and LL junior technician, V2). Postmortem examination was performed by MVF. MTT did the statistical analysis. AS-U and MTT were major contributors in writing the manuscript. All authors read an approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the European Directive for pig protection (DOUE-L-2009–80287 2009) and the Spanish legislation for animal protection in experimentation and other scientific purposes, including teaching (Real Decreto 53/2013, 2013). Expert veterinarians were in charge of caring and handling the animals. The Ethical Committee for Animal Experiments, University of Zaragoza, Spain approved this study (reference number: PI01/22). Informed consent was obtained from the owner of the animals.

Consent for publication
Not applicable.

Competing interests
SM (founder) and ML (employee) are permanent staff members from KUBUS, the company manufacturing both the calibrated catheter and the commercial ultrasound W3® mentioned in the manuscript, but this company had no significant financial contribution to this work. The rest of authors declare no conflict of interests.

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Publisher's Note

ARTICULO 2

Post-cervical compared with cervical insemination in gilts: Reproductive variable assessments

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Post-cervical compared with cervical insemination in gilts: Reproductive variable assessments

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ABSTRACT

The aim with this study was to compare cervical (CAI; 3×10^9 spermatozoa/90 mL) and post-cervical (PCAI; 1.5×10^9 spermatozoa/45 mL) artificial insemination (AI) techniques for frequency of incidences (unsuccessful or difficult probe passage, backflow, metritis and bleeding), values for reproductive variables and duration of the procedure in gilts. There were 644 gilts (255–270 days old, weighing 150 ± 5 kg) randomly assigned to PCAI (n = 320) and CAI (n = 324) groups. In total, there were 957 and 958 artificial inseminations performed in the CAI and PCAI groups, respectively (2–4 AIs/gilt). The frequency of unsuccessful or difficult PCAI probe passage/AI was 14.6% (140/958), therefore, there was a 85.7% probe passage success/AI rate (818/958). The semen backflow frequency/AI was less with PCAI than CAI (4.3% compared with 8.2%, $P < 0.001$). With the PCAI group, there were only a few cases of bleeding (11/958: 1.1% /AI) with no difference between the CAI and PCAI groups ($P = 0.224$). In gilts ($n = 72$) where there was not passage of the PCAI probe (72/320; 22.5%) there was use of CAI, (M, mixed group). For the CAI, PCAI and M groups, there were similar values for positive pregnancy diagnosis, farrowing rates and prolificacy ($P > 0.05$). The average duration for AI was shorter in the PCAI (2.34 ± 0.809 min) than CAI (4.77 ± 1.059 min) group, and it was longer in the M group (7.48 ± 2.454 min; $P < 0.050$). The PCAI procedure, therefore, is recommended for AI of gilts.

1. Introduction

Currently, most pig farms worldwide use cervical artificial insemination (CAI) in the reproductive management of gilts (Fitzgerald et al., 2008; García-Vázquez et al., 2019). For CAI, 2–3 semen doses per estrus are used, containing 2–4 × 10^9 sperm cells in a volume of 70–100 mL, stored at 17 °C for a maximum period of 3–7 days depending on the extender used.

The first field studies published using post-cervical artificial insemination (PCAI) were conducted by Watson and Behan (2002) with results being similar for PCAI and CAI. The PCAI procedure has been proposed as a new technique for depositing semen in the uterine body, therefore, there are fewer sperm numbers required without a decrease in productivity of the pork production enterprise

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(Bennemann et al., 2004; Serret et al., 2005; Roca et al., 2011). With development of the PCAI procedure, there have been new models of catheters and probes developed (Martinez et al., 2005; Kirkwood and Kauffold, 2015; Ausejo et al., 2017; Ulguim et al., 2018; Llamas-López et al., 2019).

These AI techniques differ not only in the semen deposition site but also in the sperm concentration and dose volume used for AI. The number of doses per ejaculate for CAI is limited to 20–25 (Fitzgerald et al., 2008; Wilson, 2012; Hernández-Caravaca, 2015). A minimum volume of 50 ml containing 1.5×10^9 sperm was initially considered necessary to obtain a 91.9% farrowing rate and satisfactory litter size (Behan and Watson, 2004); therefore, each individual ejaculate can be used to produce as many as 60 doses for PCAI (Hernández-Caravaca, 2015). Boars in artificial insemination (AI) centers with the greatest genetic merit for selected traits could be used in inseminating a larger number of gilts with use of PCAI (Bortolozzo et al., 2008; Sbardella et al., 2014; Knox, 2016). The main objectives for PCAI are efficient genetic progress, minimizing semen backflow during the insemination process and decreasing the time to conduct the AI procedure, without reduction of litter size and farrowing rate (Bennemann et al., 2004; Nogueira et al., 2006; Wilson, 2012; Falceto, 2018; García-Vázquez et al., 2019).

Gilt productivity is important in pork production enterprises because gilts represent 18% of the farrowing group and produce approximately 13% of the total piglets born (Ternus et al., 2017). The use of PCAI in multiparous sows is well established, but there are only a few studies where the use of this procedure has occurred in both primiparous sows and gilts. Hernández-Caravaca et al. (2012) reported that PCAI catheters used for multiparous sows could be effectively used in only 25% of the gilts. Alternatives, therefore, are needed to implement or modify PCAI in gilts. The most important limiting factor for PCAI in gilts is the smaller reproductive tract and the need for proper training of the AI technician in the use of flexible catheters (Levis et al., 2001; Ausejo et al., 2017; Hernández-Caravaca et al., 2017; Ulguim et al., 2018). The primary objective of the present study, therefore, was to compare the effects of CAI and PCAI on fertility, farrowing rate and prolificacy variables in gilts.

2. Materials and methods

2.1. Ethical declaration

This study complied with the ARRIVE guidelines (Kilkeny et al., 2010), the Council Directive, 2008/120/EC outlining minimum standards for the protection of pigs and Directive, 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures in the experiment were conducted in ways that are consistent with the precepts of animal welfare and were approved by the Committee of Ethics in Animal Experimentation of Universidad de Zaragoza (protocol No. PI31/18).

2.2. Animals

This study was conducted using the Spanish standard commercial conditions between 2016 and 2018 on three breeding sow farms (Farms 1–3) located near Caspe (Zaragoza, Northeastern Spain, Farm 1), Fonz (Huesca, Northeastern Spain, Farm 2) and Plasencia de Jalón (Zaragoza, Northeastern Spain, Farm 3). There is a description of the characteristics of these farms in Table 1. A total of 644 gilts were used in the study; gilts were 255–270 days old, weighed 150 ± 5 kg (*SD*) and had two previously detected periods of estrus. All animals were of hyper-prolific genetic lines (Farm 1: Youna, AXIOM, Azay sur Indre, France; Farm 2: Naïma, Choice Genetics France, Bruz, France; Farm 3: DanBred, DANBRED P/S, Herlev, Denmark) and were randomly assigned to the treatment group (PCAI; $n = 320$) or a control group (CAI; $n = 324$).

The number of replicates (batches of gilts submitted to AI per week) differed among farms; the number of gilts/replicate also varied within and between farms (Table 1). Gilts were fed a commercial diet twice a day. Gilts were treated with altrenogest (REGUMATE ®, Merck & Co., Inc., Kenilworth, NJ, USA) administered orally and individually for 18 days. Water was available *ad libitum*. After AI, the gilts were housed in individual pens (0.65 × 2 m) until pregnancy status diagnosis occurred (28 days after AI). After there was a positive pregnancy diagnosis, the gestating gilts were group-housed based on week when there was AI of the gilt (about 10 gilts per pen).

2.3. Sperm collection

Semen utilized in this study was obtained from 35 different Pietrain boars (UPB®, Semen Cardona; CIA San Pedro, Cuarte S.A.; CIAR, Spain) once a week using the gloved-hand technique for collections and then filtered to remove the gel. The average number of spermatozoa was assessed by using a BRAND® counting chamber BLAUBRAND® Bürker pattern (Merck & Co., Inc., Kenilworth, NJ, USA); and spermatozoa motility, agglutination and abnormalities were analyzed using the ISAS Psus® software (PROISER R + D, Paterna, Spain). Heterospermic doses from Pietrain boars were used (two boars/dose). Only ejaculates with greater than the minimum requirements (motility > 80% and total abnormalities < 25%) were used. Immediately after evaluation, each ejaculate was fully diluted in a commercial extender at 37 °C (VITASEM®, Magapor, Ejea de los Caballeros, Spain) and stored in bag doses that contained 1.5×10^9 spermatozoa per 45 ml for PCAI or 3×10^9 spermatozoa per 90 ml for CAI. Doses were stored at 15 to 18 °C for 72 h.

Table 1
Characteristics of the farms where the study was conducted; Data are reported as percentages (counts/*n*) except for gilts/replicate which is reported as mean ± SD; M: unsuccessful PCAI immediately followed by CAI.

Variable		Farm		
		1 (*n* = 123 gilts)	2 (*n* = 109 gilts)	3 (*n* = 412 gilts)
Year				
	2016	–	100 (109/109)	–
	2018	100 (123/123)	–	100 (412/412)
Number of artificial inseminations/gilt				
	2	22 (27/123)	22.9 (25/109)	–
	3	72.4 (89/123)	77.1 (84/109)	100 (412/412)
	4	5.6 (7/123)	–	–
Season of AI				
	Spring	80.5 (99/123)	100 (109/109)	29.6 (122/412)
	Summer	19.5 (24/123)	–	70.4 (290/412)
Replicates		17	4	16
Gilts/replicate		7.24 ± 3.833	22.75 ± 18.191	23.50 ± 17.143
First to second AI interval				
	12 h	30.9 (38/123)	–	90.5(373/412)
	24 h	69.1 (85/123)	–	9.5 (39/412)
Second to third AI interval				
	12 h	66.3 (59/89)	–	8.5 (5/412)
	24 h	33.7(30/89)	–	91.5 (377/412)
Third to Fourth AI interval				
	12h	–	–	–
	24h	100 (7/7)	–	–
Group				
	CAI	54.5 (67/123)	47.7 (52/109)	49.8 (205/412)
	PCAI	33.3 (41/123)	43.1 (47/109)	38.8(160/412)
	M	12.2 (15/123)	9.2 (10/109)	11.4 (47/412)

2.4. Insemination assays

Estrous detection in gilts was performed twice daily using mature boars or on the basis of standing reflex in response to human-imposed back pressure. The occurrence of estrus was defined as the standing reflex when the back pressure method was performed, as well as reddening and swelling of the vulva. There was insemination of the gilts for the first time when estrus symptoms were detected and again at intervals of 12 to 24 h throughout the duration of the period of estrus. Data in Table 1 are the number of artificial inseminations per gilt and the AI timing distributions for two of the farms; unfortunately, no data about AI timing at Farm 2 were available. The mean number of inseminations per gilt was 2.93 ± 0.29 (SD) (minimum: 2 and maximum: 4). There was only seven gilts from Farm 1 for which there was AI four times. The gilts in the control group (CAI) were inseminated in the presence of a boar, and the semen was deposited in the cranial portion of the cervix using the foam tip catheter for gilts manufactured by Magapor (Ejea de los Caballeros, Spain). For gilts in the PCAI group, insemination was conducted without the presence of a boar, using a specific PCAI probe for gilts (MAGAPLUS N®, Magapor, Ejea de los Caballeros, Spain) and, as a guide, the foam tip catheter for gilts that was manufactured by Magapor (Ejea de los Caballeros, Spain). The only methodological difference between the PCAI and CAI techniques was the use of the specific PCAI probe for gilts with doses that contained 1.5×10^9 spermatozoa per 45 mL. The duration of time required to conduct the AI procedures was recorded for 123 and 412 gilts from Farms 1 and 3, respectively; the average duration of conducting the AI was determined for each gilt. There was recording of the frequencies of unsuccessful probe passage, difficult probe passage, backflow, bleeding and metritis at insemination. No evaluation of semen backflow or bleeding volume was conducted; therefore, both semen backflow and bleeding were considered as a qualitative variable with two values being recorded (absence/presence). There were assessments for both backflow and bleeding at the time of insemination. Both CAI and PCAI procedures in every gilt were conducted by one technician per farm. For the gilts where there could not be passage of the PCAI probe, there was immediate use of CAI, and the gilts where this occurred were allocated to a third group, termed the M group (mixed group).

2.5. Return of estrus and diagnosis of pregnancy

Pregnancy was diagnosed using trans-abdominal ultrasonography (Future-1®, Inserbo, Spain) 28 days post-insemination. The return to estrus was assessed by boar stimulation or by applying back pressure to the gilts. Returns to estrus, abortions and deaths after confirmation of pregnancy were recorded by the AI technician. Values for reproductive variables (pregnancy and farrowing rates; total number of piglets born, number of live-born piglets and number of stillborn piglets and mummies per litter) were recorded for every farrowing.

2.6. Statistical analysis

All statistical analyses were performed using SPPSS v. 22. Values for qualitative variables were analyzed by cross tabulation, and percentages were compared using the Pearson's χ^2 test and, alternatively, the Fisher´s exact test (2 × 2 tables with small effective size) was used. Binomial logistic regression was applied to binary variables for pregnancy and farrowing rate: a binomial logistic regression was performed to ascertain the effects of farm, group and number of artificial inseminations on the likelihood that there would be a positive pregnancy diagnosis and whether the gilts farrowed. The General Linear Model (GML) was used for the analyses of total number of piglets born and live-born piglets/litter. The model included farm and group as fixed effects and number of artificial inseminations as a covariable; also, the total number of born (piglets/litter) was considered a covariable for the evaluation of live-born piglets/litter. Both fixed effects and covariates were maintained in the model regardless of the effect (P value). In Table 5, there are the results from use of the complete models. Data for stillborn piglets/litter and mummies/litter were analyzed using a non-parametric tests (Kruskall- Wallis test). The average amount of time for conducting AI procedures was calculated for every gilt as the sum of the duration of insemination per number of inseminations and the Breslow's test was used for comparisons between groups and farms. Differences were considered significant at $P < 0.05$.

3. Results

Data included in Table 2 are the results when the different AI procedures (CAI and PCAI) were used. There were 72 gilts for which passage of the PCAI probe could not be accomplished (72/320; 22.5%) and that were immediately submitted to CAI. These data for the gilts of the M group are shown in Table 1 and the frequency of assigning gilts to the M group did not differ among farms ($P = 0.629$). The data for distribution of gilts of the M group on the basis of the successive AI procedures are shown in Table 3.

In Table 2, there are data for the results of the total number of artificial inseminations in the CAI (957 artificial inseminations for 324 gilts) and PCAI (958 artificial inseminations for 320 gilts) groups. The PCAI was performed without problems occurring in 79.9% of the artificial inseminations (765/958). Unsuccessful or difficult PCAI probe passage only occurred in 10.6% (102/958) and 4% (38/958) of artificial inseminations, respectively. Backflow of semen occurred less frequently in PCAI than in the CAI group (4.3% compared with 8.2%; $P < 0.001$). At the time of the second AI, there was diagnosis of metritis by the presence of purulent discharge in two gilts; semen backflow and metritis were detected in only one gilt (Table 2). Frequencies of semen backflow and bleeding, backflow and metritis, and metritis and bleeding were few in both the CAI and PCAI groups, and there were no significant differences between these two groups when values for this variable were compared ($P > 0.05$). In both the CAI and PCAI groups, there were no differences among the first, second and third artificial inseminations for problems related to semen backflow, semen backflow and bleeding, semen backflow and metritis, and metritis and bleeding frequencies ($P > 0.05$). Data for the fourth AI were not included in these comparisons due to the small number of gilts for which there was a fourth AI. In the PCAI group, there were no differences among the first, second and third artificial inseminations for unsuccessful probe passage rates ($P > 0.05$). There were differences for difficult probe passage frequency in the PCAI group ($P = 0.041$) with this frequency being less for the second than third AI, but difficult probe passage frequency for the first AI did not differ when compared with that when there was a second or third AI conducted.

Data in Table 4 are for pregnancy and farrowing rates per gilt inseminated in the CAI, PCAI and M groups. For the positive pregnancy rate status, after an adjustment for the significant effect of the number of artificial inseminations, there were no differences among farms or groups. There were also no group effects on the farrowing rate.

The data for prolificacy based on total piglets born/litter and live-born piglets/litter are included in Table 5. There was no effect of the number of artificial inseminations ($P > 0.05$) on prolificacy. For the total number of piglets born/litter, there were no differences among groups, but there were differences among farms. There was an effect of total piglets born/litter on live-born piglets/litter ($P < 0.001$). For live-born piglets/litter, there were no differences among farms or groups after an adjustment for the effect of the total number of piglets born/litter ($P < 0.001$). There was no differences among groups for stillborn piglets/litter (CAI: 1.67 ± 1.764, 8.6%; PCAI: 1.59 ± 1.825, 8.3%; M: 1.37 ± 1.631, 6.8%; $P = 0.677$). For mummies/litter, there were no differences among groups (CAI: 0.41 ± 0.795, 2.1%; PCAI: 0.39 ± 0.765, 2.0%; M: 0.39 ± 0.717, 2.0%; $P = 0.937$).

There were only limited data for the average amount of time required to conduct an AI, mainly from Farm 3. At this farm, the average duration of AI was 4.77 ± 1.059 min (205 gilts), 2.34 ± 0.809 min (160 gilts) and 7.48 ± 2.454 min (47 gilts) for CAI, PCAI and M groups, respectively. The average duration of AI was less in the PCAI than CAI ($P < 0.050$) group. In the M group, the average duration of AI was longer than in the CAI group ($P < 0.050$).

Table 2
Results with use of the successive AI procedures for all gilts in the CAI and PCAI groups and the total artificial inseminations per group; Data are reported as percentages (counts/*n*); Unsuccessful probe passage: there could not be passage of the probe through the cervix; Difficult probe passage: the probe was difficult to pass through the cervix.

AI procedure		Group	
		CAI	PCAI
First	*n* (gilts)	324	320
	No problems	89.8(291/324)	79.1(253/320)
	Unsucessful probe passage	0(0/324)	10.6(34/320)
	Difficult probe passage	0(0/324)	4.4 (14/320)
	Semen backflow	9.0(29/324)	5.0(16/320)
	Semen backflow & bleeding	0.3(1/324)	0(0/320)
	Bleeding	0.9(3/324)	0.9(3/320)
Second	*n* (gilts)	324	320
	No problems	90.5(293/324)	80.6(258/320)
	Unsucessful probe passage	0(0/324)	12.8(41/320)
	Difficult probe passage	0(0/324)	1.9 (6/320)
	Semen backflow	8.0(26/234)	2.5(8/320)
	Semen backflow & metritis	0.3(1/324)	0(0/320)
	Metritis	0.3(1/324)	0.3 (1/320)
	Bleeding	0.9 (3/324)	1.9 (6/320)
Third	*n* (gilts)	307	313
	No problems	92.5(284/307)	80.2(257/313)
	Unsucessful probe passage	0(0/307)	8.3 (26/313)
	Difficult probe passage	0.3(1/307)	5.8(18/313)
	Semen backflow	7.2(22/307)	5.1(16/313)
	Bleeding	0(0/307)	0.6(2/313)
Fourth	*n* (gilts)	2	5
	No problems	50(1/2)	60(3/5)
	Unsucessful probe passage	0(0/2)	20 (1/5)
	Semen backflow	50 (1/2)	20 (1/5)
Total	*n* (AIs)	957	958
	No problems	90.8(869/957)	79.9(765/958)
	Unsucessful probe passage	0(0/957)	10.6(102/958)
	Difficult probe passage	0.1(1/957)	4.0(38/958)
	Semen backflow	8.2(78/957)	4.3(41/958)
	Semen backflow & bleeding	0.1(1/957)	0(0/958)
	Semen backflow & metritis	0.1(1/957)	0(0/958)
	Metritis	0.1(1/957)	0.1 (1/958)
	Bleeding	0.6(6/957)	1.1(11/958)

Table 3
Distribution of gilts with use of PCAI or unsuccessful PCAI immediately followed by CAI (M) in successive AI procedures; Data are reported as percentage (count/*n*). NA: third/fourth AI was not conducted.

First AI	Second AI	Third AI	Fourth AI
PCAI: 89.3 (286/320)	PCAI: 81.2 (260/320)	NA: 1.2 (4/320)	NA: 1.2 (4/320)
		PCAI: 76.3 (244/320)	NA: 75.4 (241/320)
			PCAI: 0.9 (3/320)
		M: 3.7 (12/320)	NA: 3.7 (12/320)
	M: 8.1 (26/320)	NA: 0.6 (2/320)	NA:0.6 (2/320)
		PCAI: 5.3 (17/320)	NA: 5.3 (17/320)
		M: 2.2 (7/320)	NA: 1.9 (6/320)
			M: 0.3 (1/320)
M: 10.7 (34/320)	PCAI: 6.0(19/320)	PCAI: 5.7 (18/320)	NA: 5.3(17/320)
			PCAI: 0.3 (1/320)
		M: 0.3 (1/320)	NA: 0.3 (1/320)
	M: 4.7 (15/320)	NA: 0.3 (1/320)	NA: 0.3 (1/320)
		PCAI: 2.5 (8/320)	NA: 2.5 (8/320)
		M: 1.9 (6/320)	NA: 1.9 (6/320)

4. Discussion

As reported by Bortolozzo et al. (2015), there has been a wide range in volume (10–85 mL) and number of sperm ($0.1–4 \times 10^9$) used for PCAI. In the present study, PCAI and CAI techniques were different than those in previous studies not only in semen deposition site but also in sperm concentration and dose volume. In the present study, therefore, there were comparisons of the

Table 4
Pregnancy and farrowing rate per gilt inseminated in CAI, PCAI and M groups; Data are reported as percentages (counts/*n*); M: unsuccessful PCAI immediately followed by CAI; Pregnancy rate: proportion of inseminated females that were pregnant; Farrowing rate: proportion of inseminated females that farrowed.

Variable	Group			Effect		
	CAI (n = 324 gilts)	PCAI (n = 248 gilts)	M (n = 72 gilts)	Farm P	Group P	Number of AI P
Pregnancy rate	91.4 (296/324)	92.3 (229/248)	94.4 (68/72)	0.371	0.673	0.048
Farrowing rate	85.8 (278/324)	88.7 (220/248)	93.1 (67/72)	0.138	0.213	0.157

Table 5
Total number of piglets born and live-born piglets of gilts with use of different techniques for artificial insemination; Data are reported as mean ± SD; M: unsuccessful PCAI immediately followed by CAI.

Variable	Group			Effects			
	CAI (n = 278 litters)	PCAI (n = 220 litters)	M (n = 67 litters)	Group P	Farm P	Number of AI P	Total born P
Total piglets born/litter	18.28 ± 4.430	18.46 ± 4.380	17.79 ± 4.413	0.295	< 0.001	0.122	
Live-born piglets/litter	16.20 ± 3.938	16.51 ± 4.158	16.03 ± 3.618	0.487	0.490	0.824	< 0.001

effectiveness of both the specific PCAI probe used for gilts and small sperm concentration and dose volume.

The farms differed for important factors in this study (year, number of AIs/gilt, season, replicates, gilts/replicate, AI timing), although there were no significant differences for CAI, PCAI and M percentages. These factors were associated with farms and, therefore, could not be included in the GLM as independent factors, but the effects were grouped into the factor “farm”.

The use of PCAI represents an important aspect of the developments in reproductive biotechnology that have occurred on swine farms in recent decades. The use PCAI has increased worldwide, although application is limited mainly to multiparous sows. Since 1959, when Hancock (1959) described and used nonsurgical intrauterine insemination for the first time in multiparous sows, there has been use of several types of instruments, such as endoscopes, catheters or probes and pipettes for this purpose. There are certain limitations, including the lack of suitability for application in Landrace × Large White gilts (Hernández-Caravaca et al., 2017; Llamas-López et al., 2019).

In the present study, the small frequencies of unsuccessful or difficult PCAI probe passage per AI ([102 + 38]/958 = 14.6%) resulted in a percentage of probe passage success of 85.4% per AI (818/958), a relatively greater percentage when compared to the percentages that are generally reported. Usually, in Landrace × Large White lines the percentages of success in probe passage are 95% in multiparous and 85% in primiparous (Sbardella et al., 2014; Bortolozzo et al., 2015; Hernández-Caravaca et al., 2017). The results in the present study are inconsistent with the recommendation not to use PCAI for gilts (Levis et al., 2001; Dallanora et al., 2004 Cambourough 22 line from Agroceres PIC, Rio Claro, São Paulo, Brazil), but are consistent with results of Sonderman (2016) and Ternus et al. (2017) where it was concluded that PCAI can be used in Landrace × Large White gilts without compromising enterprise pork production efficiency. In recent years, there was development of new catheters and probes to prevent injuries to the gilts while conducting AI procedures and to simplify the use of the PCAI procedure on commercial farms (García-Vázquez et al., 2019).

Most of these studies were conducted with Landrace × Large White gilts, but no detailed information about age and weight was available. Lines used in the present study had a Large White and/or Landrace ancestry and it is important to recognize that the specific cross schemes used to produce commercial gilts could lead to different animal sizes. The standard age for first insemination is usually 220–230 days of age; thus, there are apparently only minor differences in weight, therefore, weight differences could not account for the differences in results in the present compared with those in some previous studies. There was use of a specific PCAI probe for gilts (MAGAPLUS N®, Magapor, Ejea de los Caballeros, Spain) in the present study and, as a guide, the foam tip catheter was used for PDAI in the present study (Magapor; Ejea de los Caballeros, Spain). It is believed that the use of these devices in the present study that were not used in some of the previous studies could explain for the result differences between the present and previous studies.

In the PCAI group, the frequency of semen backflow per AI was less than in the CAI group. Ausejo et al. (2017); Dominiek et al. (2011) and Hernández-Caravaca et al. (2017) reported similar results in both multiparous sows and gilts (Landrace × Large White lines). Semen backflow occurs due to many factors, but it is usually explained by the AI procedure being performed incorrectly thus causing difficulty of passage and torsion of the internal catheter that results in the backflow of the semen (Ausejo et al., 2017; Ternus et al., 2017; García-Vázquez et al., 2019).

Semen backflow, semen backflow and bleeding, semen backflow and metritis, and metritis and bleeding rates did not seem to be affected by the number of artificial inseminations performed on individual gilts in the present study. For the PCAI group, only a very small number of bleeding cases were observed (11/958: 1.1% per AI) and there were no differences between the CAI and PCAI groups (P = 0.224). Bleeding would be related to the animal, rather than to the number of artificial inseminations. Serret et al. (2005);

Rozeboom et al. (2014) and Llamas-López et al. (2019) advised against the use of PCAI in Landrace × Large White gilts because of the small size of the reproductive tract in gilts, the use of the PCAI procedure was considered to cause cervical injuries. Results from the present study, however, indicate the use of a specific probe by a qualified technician does not result in severe lesions in the cervix of gilts, confirming the previously reported results of Ausejo et al. (2017).

Although there were differences between farms, the average duration taken to conduct the AI procedure per gilt was longer in the CAI than PCAI group. The use of a specific PCAI probe and smaller dose volume likely contributes to this difference. Similar results were reported by Ternus et al. (2017).

The differences for difficult probe passage frequency among successive artificial inseminations could not be clearly explained by the increase or decrease in this rate as the number of artificial inseminations increased. The effect of the number of artificial inseminations per gilt was significant only for the positive pregnancy diagnosis-rate status, but after an adjustment for this effect, there was no effect for farm or group.

There was an effect of farm on total number of born piglets/litter. Effects of year, number of artificial inseminations/gilt, season, replicates, gilts/replicate, AI timing and farm were grouped as a “farm” effect; therefore, it was not possible to assess the specific effect of each factor. In the present study, the most important effect on live-born piglets/litter, stillborn piglets/litter and mummies/litter was consistent with the finding for total number of born piglets/litter; after an adjustment for this significant effect ($P < 0.001$), there was no effect of farm or group.

Values for reproductive variables in the present study are consistent with those previously reported by Sonderman (2016) and Ternus et al. (2017) with there being no differences for pregnancy and farrowing rates between the CAI and PCAI groups. There were also no differences in values for prolificacy variables (total born, live-born and stillborn piglets and mummies/litter) between these two groups and there have been similar results reported by Fitzgerald et al. (2008); Hernández-Caravaca et al. (2012) and Hernández–Caravaca (2015) in multiparous sows and Sbardella et al. (2014) in primiparous gilts. Results from the present study indicate that PCAI does not compromise the reproductive performance in gilts.

5. Conclusions

In gilts, the percentage of PCI conducted without problems being incurred was 79.9% per AI (765/958) with small percentages for both unsuccessful probe passage (10.6%, 102/958) and difficult probe passage (4.0%, 38/958). The frequency of semen backflow, metritis and bleeding was also small (global frequency: 5.5%, 43/958). The use of the PCAI technique, performed with semen doses containing 1.5×10^9 sperm cells per 45 mL, resulted in reproductive and prolificacy performances similar to those observed with traditional CAI (semen doses 3×10^9 sperm cells per 90 mL). There, therefore is recommendation for use of the PCAI procedure for AI of gilts.

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Declarations of Competing Interest

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ARTICULO 3

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

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REVIEW

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

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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).

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

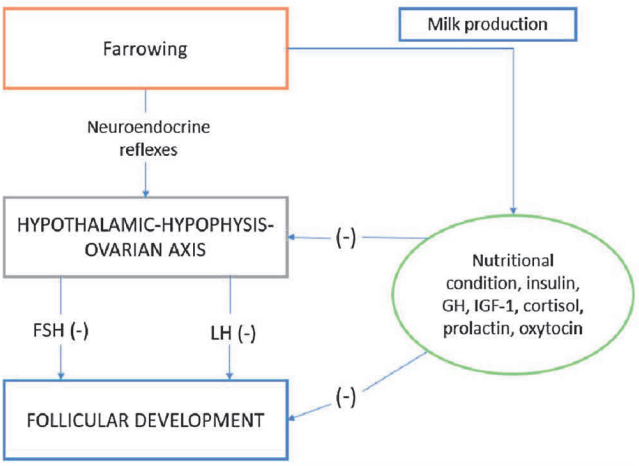

FIGURE 1 Hypothalamic–hypophysis–ovarian axis in sows after farrowing and during lactation. The symbol (–) indicates negative feedback.

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 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).

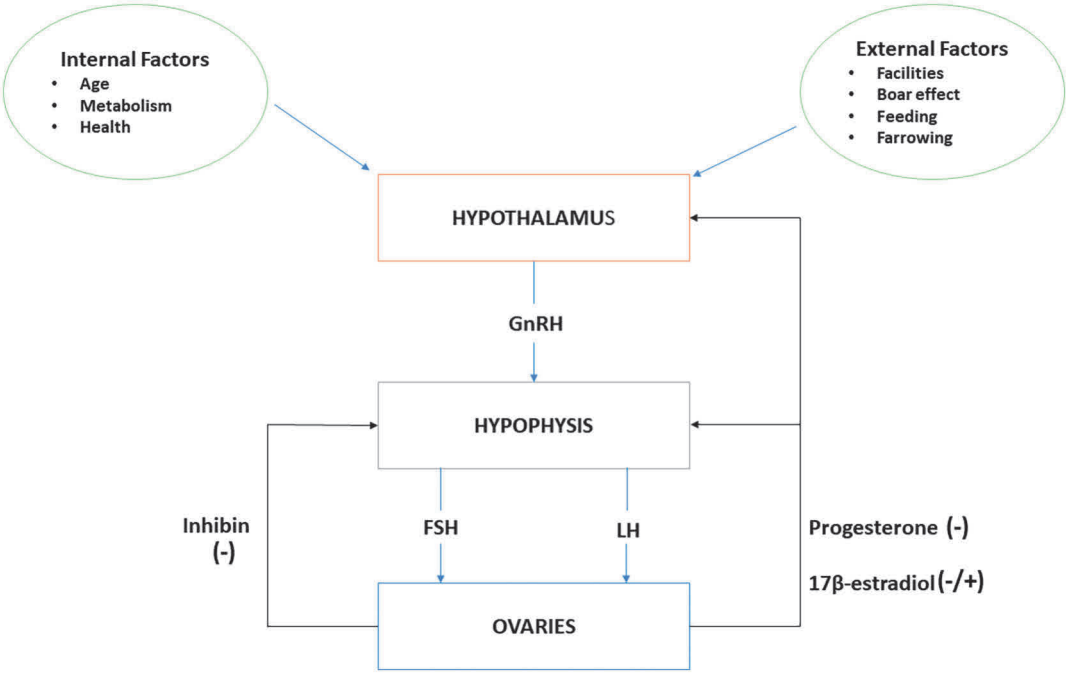

FIGURE 2 Effect of lactation on the hypothalamic–hypophysis–ovarian axis.

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

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)	no of 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).

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.

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ARTICULO 4

Single Fixed-Time Post-Cervical Insemination in Gilts with Buserelin

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Article

Single Fixed-Time Post-Cervical Insemination in Gilts with Buserelin

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Simple Summary: Current protocols for gilts recommend the deposit of two/three semen doses (2–4 × 10^9 sperm/dose) by cervical artificial insemination (CAI), 12–24 h after estrus detection. If ovulation were predictable, gilts could be bred only once using fixed-time artificial insemination (FTAI). Using a specific catheter makes the postcervical deposition of semen possible (PCAI). This work explored the use of combining FTAI-PCAI with buserelin in gilts. In the control group (C; $n = 240$), gilts were inseminated twice (8 and 12 h from estrus onset). Gilts in the treatment group (T; $n = 226$) received buserelin (10 µg, intramuscular) 120 h after altrenogest treatment (18 d) and one single PCAI 30–33 h after buserelin administration. No significant differences were found in reproductive and production performance between groups ($p > 0.05$). Piglets' birth weight was greater in the T group ($p < 0.001$). Estrus duration was significantly shorter in the T group ($p < 0.001$). Delivery batch length significantly differed depending on the season ($p < 0.05$); both groups only differed significantly in spring ($p = 0.018$), with a shorter duration in the T group. This new FTAI-PCAI protocol with buserelin is recommended in gilts, helping with optimization of genetic diffusion, boars, and semen doses.

Abstract: Current protocols for gilts recommend the deposit of multiple semen doses in the cervix each 12–24 h after estrus detection. Our objectives were: (1) to determine the effect of buserelin and a single fixed-time artificial insemination using the new post-cervical artificial insemination technique (FTAI-PCAI) on reproductive and productive performance in gilts, and (2) to compare this protocol with conventional estrus detection and double PCAI without hormonal induction. In the control group (C; $n = 240$), gilts were inseminated twice (8 and 12 h from estrus onset). Gilts in the treatment group (T; $n = 226$) received buserelin (10 µg, intramuscular) 120 h after altrenogest treatment (18 d) and one single PCAI 30–33 h after buserelin administration. The groups did not differ in reproductive and production performance ($p > 0.05$). The T group showed greater piglet birth weight and shorter estrus duration ($p < 0.001$). Delivery batch length differed significantly depending on the season ($p < 0.05$); the shortest length corresponded to autumn. Both groups only differed significantly in spring ($p = 0.018$), with a shorter length in the T group. This new FTAI-PCAI protocol with buserelin is recommended in gilts, helping with optimization of genetic diffusion, boars, and semen doses.

Keywords: fixed-time insemination; buserelin; gilt; post-cervical

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1. Introduction

The main aim of artificial insemination (AI) is to deposit enough viable sperm in the appropriate place of the female genital tract at the optimal moment relative to ovulation. For gilts, current protocols recommend the deposit of multiple semen doses (two or three) in the cervix each 12–24 h after estrus detection (2–4 $\times$ 10^9 sperm at 60–100 mL per dose, stored at 17 °C for a maximum period of 3–7 days, depending on the extender) [1,2]. Reasons for repeated AI lie in the brief viability of both oocytes and spermatozoa in the gilt reproductive tract and the difficultly of exactly predicting ovulation during estrus [3].

The post-cervical artificial insemination (PCAI) procedure was proposed as a new technique for depositing semen in the uterine body, as an alternative to cervical AI (CAI). PCAI and CAI differ not only in the semen deposition site but also in the sperm concentration and dose volume used for AI [2,4,5]. PCAI promotes efficient genetic progress, minimizes semen backflow during the insemination process, and decreases the time to conduct the AI procedure, without reduction in litter size and farrowing rate [2,4,6,7]. The suitability of PCAI in gilts has been previously demonstrated [2,8].

Fixed-time artificial insemination (FTAI) involves one single semen dose applied within a period of 0–24 h before ovulation [9]. A better understanding of regulation mechanisms for follicular development and ovulation has enabled new perspectives on ovulation control in gilts, and therefore for FTAI development [10]. In gilts, both estrus duration and the estrus onset-ovulation interval are highly variable; therefore, controlling ovulation timing is needed [1,9,11].

Gonadotropin-releasing hormone (GnRH) analogues, luteinizing hormone (LH), and human chorionic gonadotropin (hCG) have been efficiently used to induce ovulation in weaned sows and gilts, always with CAI [1]. Several studies have recommended the combined use of porcine LH (pLH) plus equine chorionic gonadotropin (eCG) with single or double PCAI in sows [12,13]. Additionally, one single pLH application at the estrus onset has shown good results in sows with either single [14] or double PCAI [15,16]. Several GnRH agonists have been used as ovulation inductors, as a previous step to various AI techniques. Licerelin with CAI has been applied to sows [17]. Buserelin with PCAI has been used in sows [18], while buserelin with double CAI has been applied in gilts [19]. In both sows and gilts, goserelin with CAI has been used [11]. Triptorelin with PCAI has been used in sows [20,21], and more recently, a single-FTAI triptorelin protocol with CAI was used in gilts [22].

Furthermore, good reproductive results were obtained in gilts with an altrenogest treatment followed at 115–120 h after completion by buserelin administration, which enabled FTAI-CAI to be performed 30–33 h later [19]. The simplest protocols with single hormonal application showed effectiveness in synchronizing ovulation and reducing the number of inseminations [20,23]. This reduction in cost and labour of hormonal protocols could ease single FTAI use in routine AI protocols in gilts. Rodrigues [24] compared two different protocols to synchronize ovulation before FTAI-CAI (eCG vs. Triptorelin acetate) in gilts from an experimental farm; these protocols were based on procedures previously used in multiparous sows, and farrowing rates and litter sizes were lower than those obtained with the current AI technique. Therefore, more research is needed for the establishment of new protocols to improve reproductive performance in gilts, considering the differences between farms.

In this line of thought, the objective of this work was twofold. The first objective of the present study was to determine the effect of buserelin (as a synchronization protocol) and a single FTAI (using the new PCAI technique) on reproductive and productive performance when used in gilts from a commercial farm. The second objective was to compare the effects of this protocol with those of conventional estrus detection and double PCAI without hormonal induction on reproductive and productive performance in these gilts.

2. Materials and Methods

2.1. Ethical Declaration

This study complied with the ARRIVE guidelines [25], Council Directive 2008/120/EC outlining minimum standards for the protection of pigs, and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures in these experiments were conducted consistently with the precepts of animal welfare and approved by the Committee of Ethics in Animal Experimentation of Universidad de Zaragoza (protocol No. PI35/21NE).

2.2. Animals

This study was conducted according to the Spanish standard commercial swine production on a breed farm located near Huesca (Capella, north-eastern Spain). A total of 482 gilts were used in the study; these gilts were 255–270 days old, weighed 150 $\pm$ 5 kg (SD), and had two previously detected periods of estrus. All animals belonged to a hyper-prolific genetic line (DanBred, DANBRED P/S, Herlev, Denmark).

Twice a day, gilts were fed a commercial diet (3 kg/day) containing 3200 kcal EM/kg, 14% PB, and 0.7% digestible lysine. All gilts were treated with altrenogest (REGUMATE®, Merck & Co., Inc., Kenilworth, NJ, USA) administered orally and individually for 18 days. Water was available ad libitum. After AI, the gilts were housed in individual pens (0.65 $\times$ 2 m) until pregnancy status diagnosis occurred (24 days after AI).

Semen doses used in this study were obtained from 49 different Pietrain boars, belonging to an insemination center, (Semen Costean; CIA, Costean, Spain), located several Km far from the farm where the gilts were housed. Boars received a specific diet; 2.6–3.0 kg for pig males between 200 and 300 kg, containing 3000 kcal EM/kg and 0.5% digestive lysine.

2.3. Sperm Collection

Semen doses used in this study were obtained once a week using the gloved-hand technique for collections and then filtered to remove the gel. The average number of spermatozoa was assessed using a BRAND® counting chamber BLAUBRAND® Bürker pattern (Merck & Co., Inc., Kenilworth, NJ, USA). Spermatozoa motility, agglutination, and abnormalities were analyzed using the AndroVision® software 12500/0000 (Minitube, Tiefenbach, Germany). According to current protocols at the insemination center, we were only provided with ejaculates complying the minimum requirements (motility > 80% and total abnormalities < 20%). Immediately after evaluation, each ejaculate was fully diluted in a commercial extender at 37 °C (VITASEM®, Magapor, Ejea de los Caballeros, Spain). The ejaculates were combined in heterospermic doses and stored in bag doses containing 1.5×10^9 spermatozoa per 60 mL for PCAI. These heterospermic doses were identical in each insemination batch for both groups considered in this study. Doses were stored at 15 to 18 °C for 72 h.

2.4. Oestrus Detection and Duration

After Regumate treatment, gilts were randomly assigned to control (C; n = 244) and treatment (T; n = 238) groups. Estrus detection was performed twice daily (8:00 and 13:00) in both groups, using mature boars and the standing reflex in response to back pressure, as well as the presence of reddening and swelling of the vulva, according to the sequence characterized by Signoret [26]. Once estrus was detected in a gilt, the sign + was marked on its back, and the day and time were recorded. The estrus duration was the interval (h) from the establishment to the stop of the standing reflex. When no estrus signs were present, gilts were considered in anestrus and removed from the experiment.

2.5. Insemination Assays

In the C group, 240 gilts were inseminated for the first time 8 h after estrus onset and again 12 h later, during the estrus period. Both inseminations were carried out by means of the PCAI technique.

Gilts in the T group received 10 µg of intramuscular buserelin (Porceptal®, MSD, Salamanca, Spain) 120 h after the last altrenogest dose. Estrus detection was performed 30–33 h later. Only gilts with standing reflex and reddening and swelling of the vulva were prepared for single-dose PCAI; 226 gilts were inseminated in the T group.

Gilts in C group were inseminated between 8 and 8:30 and between 13:30 and 14:00; inseminations of gilts in T group were carried out between 13: 00 and 14:00. Semen doses were obtained less than 24 h before use for both first insemination of C group and unique insemination of T group, and therefore they have been obtained less than 48 h before its use in second insemination of C group. During transport and in the farm, semen doses were kept at 17 °C.

All inseminations were carried out between December 2019 and August 2020 (eight batches for each group). In both the C and T groups, PCAI was conducted in the same way; without the presence of a boar, we used a specific PCAI probe for gilts (MAGAPLUS N®, Magapor, Ejea de los Caballeros, Spain) and, as a guide, the foam tip catheter for gilts that was manufactured by Magapor (Ejea de los Caballeros, Spain). Dose concentration was always 1.5×10^9 spermatozoa per 60 mL. When the PCAI probe could not pass through the cervix (unsuccessful probe passage), gilts in either of the two groups were submitted to CAI and removed from the study; from then on, only 194 and 203 gilts were considered for the C and T groups, respectively. Occurrences of AI problems such as difficult probe passage, bleeding, semen backflow, and metritis were individually recorded. Figure 1 shows the study design.

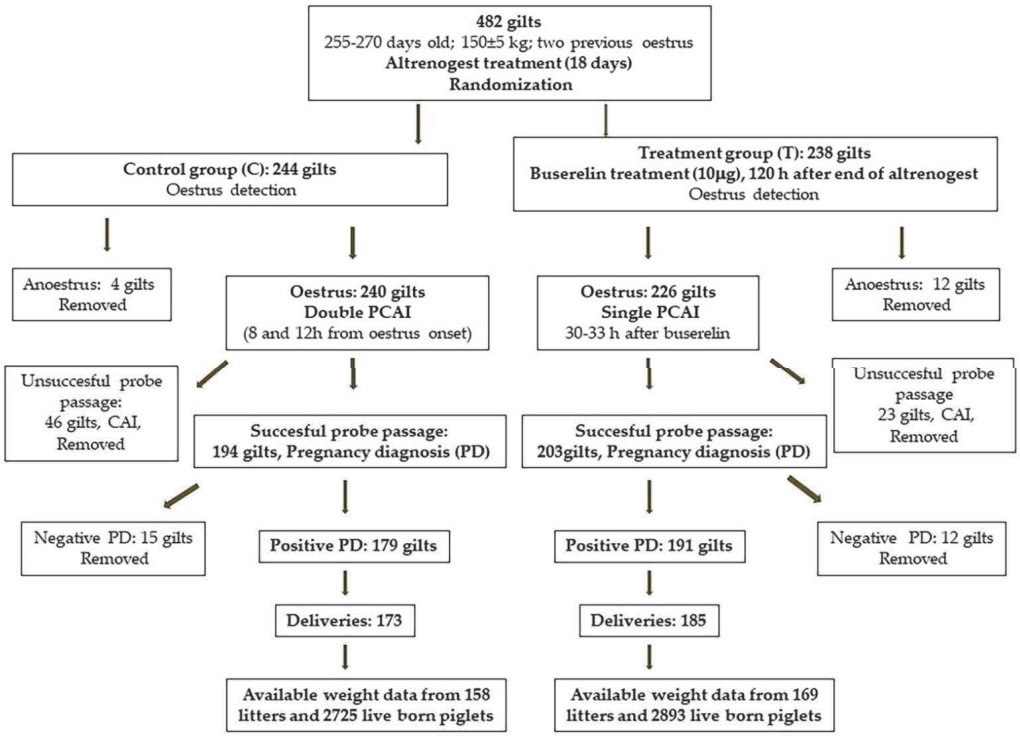

Figure 1. Study design. PCAI: post cervical artificial insemination; CAI: cervical artificial insemination.

2.6. Pregnancy Detection and Return to Oestrus

Trans-abdominal ultrasonography (Future-1®, Inserbo, Spain) 28 days after insemination was used for pregnancy diagnosis. The pregnancy rate was calculated as the proportion of inseminated females that were pregnant. After positive diagnosis, the AI technicians recorded returns to estrus, abortions, and deaths. Reaction to boar stimulation or back pressure evidenced the return to estrus.

2.7. Farrowing and Litter Parameters

The farrowing rate was calculated as the proportion of inseminated females that farrowed. Additionally, the frequencies of dystocia, total piglets born/litter, live-born piglets/litter, stillborn piglets/litter, and mummies/litter were recorded. Gestation duration and weaning-estrus (days) were recorded for every farrowing gilt. The delivery batch duration was measured as the period (days) between the first and last deliveries in the batch.

Live-born piglets were individually weighed (kg) within 24 h after birth and before cross-fostering. In this operation, a calibrated scale was used (ECE 50K-2N, Kern & Sohn GmbH, Balingen, Germany). Litter weight was also estimated (kg).

2.8. Statistical Analysis

All statistical analyses were performed using SPSS v. 26. Categorical variables were analyzed by cross-tabulation, and percentages were compared using Pearson's χ^2 test. Quantitative variables were summarized as mean ± standard error (SE). Coefficient of variation (CV = standard deviation/mean) was calculated for every litter, as an estimation of litter homogeneity. Summaries for time variables (durations and intervals) also included median ± SE.

ANOVA was used to compare the C and T groups (fixed effects) for total piglets born/litter. The ANOVA model for live-born piglets/litter and individual and litter weight within 24 h after birth and before cross-fostering also included total piglets born/litter as a covariate. A non-parametric test (Mann–Whitney U test) was used to compare the C and T groups for stillborn piglets/litter, mummies/litter, and CV. Kaplan Meier's survival analysis was applied to time variables: Breslow's test was used for comparing the C and T groups. Differences were considered significant at $p < 0.05$.

3. Results

Table 1 shows the AI results for every inseminated gilt (240 and 226 individuals in the C and T groups, respectively). Probe passage was never forced. Difficult passage means that difficulties occurred but finally the probe managed to gently pass through the cervix. Differently, unsuccessful probe passage means probe passage was impossible. As stated in the Material and Methods section, gilts in the C group were inseminated twice; unsuccessful probe passage in one of the AIs was enough for a gilt to be removed from the study. The C and T groups significantly differed in the proportion of gilts showing successful probe passage (194/240: 80.8% vs. 203/226: 89.8% in the C and T groups, respectively, $p = 0.006$). Frequencies for other problems (difficult probe passage, bleeding, semen backflow, metritis) did not differ significantly between groups in the animals that remained in the study (42/194: 21.9% and 30/203: 14.8% in the C and T groups, respectively).

Table 1. Problem frequencies for every inseminated gilt. Data are reported as percentages and, in brackets, count/*n*, where *n*: number of gilts.

Group	AI1		AI2	
	Problem	**% (Count/*n*)**	**Problem**	**% (Count/*n*)**
C	None	77.5 (186/240)	None	78.8 (189/240)
	Unsuccessful probe passage	13.3 (32/240)	Unsuccessful probe passage	10.5 (25/240)
	Difficult probe passage	6.2 (15/240)	Difficult probe passage	9.1 (22/240)
	Semen backflow	1.3 (3/240)	Semen back flow	0.4 (1/240)
	Metritis	0.4 (1/240)	Metritis	0.8 (2/240)
	Bleeding	1.3 (3/240)	Bleeding	0.4 (1/240)
T	None	76.5 (173/226)		
	Difficult probe passage	11.5 (26/226)		
	Unsuccessful probe passage	10.2 (23/226)		
	Metritis	0.9 (2/226)		
	Bleeding	0.4 (1/226)		
	Metritis and bleeding	0.4 (1/226)		

Table 2 presents data regarding the reproductive and production performance of gilts in both the C and T groups.

Table 2. Reproductive and production performances for studied gilts. Data are reported as percent-ages and count/*n* (for pregnancy rate, farrowing rate (where *n*: number of gilts), and dystocia (where *n*: number of deliveries); mean ± SE (standard error) is shown for total piglets born/litter, live-born piglets/litter, stillborn piglets/litter, and mummies/litter.

Variable	**C**	**T**	***p*-Value**
	(*n* = 194 Gilts)	**(*n* = 203 Gilts)**	
Pregnancy rate	92.3 (179/194)	94.1 (191/203)	0.602
Farrowing rate	89.2(173/194)	91.1 (185/203)	0.627
Dystocia	19.1 (33/173)	20.0 (37/185)	0.931
Total piglets born/litter	18.52 ± 0.291	18.12 ± 0.284	0.339
Live-born piglets/litter	17.38 ± 0.274	17.04 ± 0.277	0.984
Stillborn piglets/litter	0.90 ± 0.179	0.80 ± 0.108	0.356
Mummies/litter	0.23 ± 0.041	0.31 ± 0.052	0.396

No significant differences were found in any case ($p > 0.05$).

Data regarding estrus, gestation, and weaning-estrus are shown in Table 3.

Table 3. Estrus, gestation, and weaning-AI interval duration for the studied gilts. *n*: number of gilts.

Variable	**C (*n* = 194 Gilts)**		**T (*n* = 203 Gilts)**		***p*-Value**
	Mean ± SE	**Median ± SE**	**Mean ± SE**	**Median ± SE**	
Estrus duration (h)	62.613 ± 0.235	64.000 ± 0.103	59.542 ± 0.301	59.000 ± 1.039	<0.001
Gestation duration (d)	113.133 ± 0.470	115.000 ± 0.231	114.595 ± 0.289	115.000 ± 0.150	0.303
Weaning-estrus (d)	6.089 ± 0.396	5.000 ± 0.149	7.119 ± 0.406	5.000 ± 0.133	0.787

Significant differences between groups were only detected for estrus duration, which was significantly shorter in the T group ($p < 0.001$). According to median values, 50% of gilts in the T group finished estrus in 59 h, versus 64 h for the C group.

Table 4 presents data regarding the delivery batch duration of considered gilts. Deliveries occurred in eight different batches throughout 2020: three batches in spring (21 March–20 June), one batch in summer (21 June–20 September), and five batches in autumn (21 September–20 December).

Table 4. Delivery batch duration of studied gilts in total, T, and C groups. SE: standard error, *n*: number of deliveries.

Season	Total			C			T			*p*-Value
	n	Mean ± SE	Median ± SE	*n*	Mean ± SE	Median ± SE	*n*	Mean ± SE	Median ± SE	
Spring	112	2.830 ± 0.100 ^{A}	3.00 ± 0.168	57	3.053 ± 0.143 ^{b}	3.00 ± 0.247	55	2.600 ± 0.134 ^{a}	2.00 ± 0.189	0.018
Summer	29	3.448 ± 0.202 ^{B}	4.00 ± 0.226 ^{B}	14	3.500 ± 0.327	3.00 ± 0.624	15	3.400 ± 0.254	4.00 ± 0.161	0.856
Autumn	216	2.546 ± 0.079 ^{C}	2.00 ± 0.088	101	2.5045 ± 0.127	2.00 ± 0.173	115	2.548 ± 0.098	2.00 ± 0.102	0.747

^{a,b}: different letters in the same row mean significant differences ($p < 0.05$). ^{A,B,C}: different letters in the same column mean significant differences ($p < 0.05$).

Delivery batch length differed significantly depending on the season; in summer, duration was longer than in autumn ($p < 0.001$) and spring ($p = 0.006$), and a significant difference ($p = 0.020$) was also found for spring versus autumn. According to median values, 50% of gilts gave birth in the two first days of the delivery batch in autumn, but this accumulate percentage was not reached until the third day in spring and the fourth day in summer. When the C and T groups were compared within season, they only differed significantly in spring ($p = 0.018$), with a shorter duration in the T group; a difference of one day was observed in the median value, in favor of the T group.

Table 5 indicates piglets' weight within 24 h after birth and before cross-fostering (individual and litter weight, CV).

Table 5. Piglets' weight within 24 h after birth and before cross-fostering (individual and litter weight, CV: coefficient of variation). SE: standard error, *n*: number of data.

Variable	T		C		*p*-Value
	n	Mean± SE	*n*	Mean± SE	
Individual weight (kg)	2893	1.3429 ± 0.00329	2725	1.3199 ± 0.00298	<0.001
Litter weight (kg)	169	22.9294 ± 0.42655	158	22.7570 ± 0.42846	0.186
CV	169	6.7141 ± 0.41948	158	7.5326 ± 0.38916	0.092

Significantly greater values for individual weight were found for the T group ($p < 0.001$), but no significant differences were found for litter weight ($p > 0.050$). Low CV values were found for both the C and T groups. No significant differences for CV were detected ($p = 0.092$); however, both 25th and 50th percentiles were lower in the T group (1.45 and 5.77, respectively) than in the C group (3.55 and 7.27, respectively), pointing to a tendency for more homogeneous litters in the T group.

4. Discussion

The use of PCAI has increased worldwide. PCAI has been widely studied in multiparous and primiparous sows [27–36]. In recent years, there have been many PCAI trials in nulliparous gilts [2,8,31,37–39], with different results. Therefore, more studies and research are needed to improve the PCAI technique in gilts. To the best of our knowledge, this is the first study about using PCAI in a single-dose FTAI for gilts.

The main objective of applying the PCAI technique in gilts is the successful insertion of a cannula through the cervix, hampered by the small size of the reproductive tract. In the present study, the success of the probe passage through the cervix reached 80.8% and 89.8% for the C and T groups, respectively ($p < 0.05$), in contradiction with previous studies that advised against PCAI for gilts [8,31,37]. However, these results were similar to those obtained by other researchers [38,39], including a previous work of ours, where we recommended PCAI for Landrace × Large White gilts without compromising reproductive parameters' efficiency [2]. The frequencies of problems during AI (difficult probe passage, semen backflow, metritis, and bleeding) did not differ significantly between groups (C: 21.9% and T: 14.8%; $p > 0.05$). These results suggest that the use of a specific probe by a qualified technician did not result in severe lesions in the cervix of gilts, confirming previous reports [40]. Post-cervical insemination provides a number of advantages, such as a reduced sperm number requirement, and therefore, it can be used in new FTAI protocols

to accelerate genetic improvement programs [7]. On the other hand, although PCAI was used in both groups, each gilt was inseminated twice in the C group and only once in the T group; therefore, the probability of unsuccessful probe passage decreased in the T group, as expected.

Several analogues of GnRH have been used in sows and gilts as ovulation inductors, and their effects on reproductive parameters have been studied. D-Phe6-LHRH, a luteinizing hormone-releasing hormone antagonist, is a GnRH + hCG analogue (Gonavet®, Berlin-Chemie, Berlin, Germany) that produced variable reproductive results in gilts and sows [41]. A trial using eCG (Folligon®, Intervet, Whitby, ON, Canada) and double FTAI protocols only evaluated LH, FSH levels, ovulation rate, and quality in gilts [42], in weaned sows [11], and in both gilts and sows [10]. Much of this information is limited and the methodology quite variable. Differences in the approach and effectiveness appear to result from alternative use of gonadotropins, such as eCG, to synchronize follicle development, use of induction hormone GnRH or one of its agonists (pLH or hCG), the dosage and time of administration for the induction hormone, and the time of AI following induction [15,22]. Another GnRH analogue, lGnRH-III (peforelin, Maprelin® XP10, Veyx-Pharma, Schwarzenborn, Germany) did not show significant differences in reproductive parameters and litter size when compared with untreated gilts., even though peforelin seemed to have a positive effect on follicle growth [11,43,44].

Only a few studies used the GnRH analogue buserelin in FTAI protocols for gilts. Martinat-Botté [19] used buserelin as to effectively stimulate LH secretion to induce ovulation in gilts, followed by applying a double FTAI-CAI protocol (3×10^9 spermatozoa). Pregnancy, farrowing rate, and litter size were similar to our results from a single FTAI-PCAI buserelin protocol in gilts.

Most recently, the intravaginal application of a gel containing the GnRH analogue triptorelin (OvuGel®; Elanco, Guelph, ON, Canada) was promoted in both sows and gilts [1,22,45,46]. Use of triptorelin is recommended in weaned sows. They received 200 Ug of triptorelin either 96 h after weaning or at estrus detection and were subsequently inseminated with a double FTAI-PCAI [1,21] and a single FTAI-PCAI [15,47]. The use of 100–400 μg triptorelin with FTAI in gilts at 120 h after Regumate treatment obtained great results for ovulation induction, but the optimal timing of triptorelin administration after Regumate protocol might not yet have been identified in gilts [22]. In contrast, a single FTAI-CAI triptorelin protocol applied 120 h after Regumate treatment in gilts showed a farrowing rate lower than in the control group (80.3% versus 89.5%) [24]. Future research must be conducted using FTAI triptorelin protocols in gilts.

Estrus duration was shorter in gilts treated with buserelin ($p < 0.001$). Ulguim [15] did not find a significant effect of intramuscular pLH on estrus duration in gilts, but when pLH was administered by the vulvar submucosa route, estrus duration significantly decreased with respect to the control group ($p < 0.05$). Martinat-Botté [19] showed that double FTAI with buserelin in gilts can reduce estrus duration ($p < 0.05$). Driancourt [18] and Pearodwong [48] used buserelin in sows, and no significant modification was found in both estrus and weaning-AI interval duration. Similar results were found when using peforelin in gilts [43,44].

The proposed protocol, buserelin plus single FTAI-PCAI, is aimed at breeding synchronization through ovulation induction and the efficient use of semen doses with the PCAI technique. Therefore, farrowing would be expected to be grouped in a short period. The advantages of all sows farrowing close together includes improved labour efficiency in the farrowing room due to easier cross-fostering, better supervision of farrowing, and efficient processing of litters [15,24]. Our results showed a significant effect of season on delivery batch duration, with shorter values in autumn.

The sow has an important physiological basis inherited from the reproductive seasonality pattern of its ancestor, the wild boar. The European wild boar (Sus scrofa) is considered a short day breeder due to its circadian secretion pattern of melatonin [49]; in this way, wild boar piglets are born in autumn, when food availability is high. Management factors (tem-

perature, light, and feed) control the functioning of the hypothalamic-hypophysis-ovarian (HHO) axis and select autumn as the best time of year for reproduction. During summer and early autumn, the reproductive seasonality syndrome is frequent; this syndrome is caused by an imbalance of the HHO axis that affects follicular development and results in decreased reproductive parameters and increased percentage of anestrus gilts in [50].

Usually, female wild boars have ovarian activity only from November to April [51]. This shared pattern with its domestic descendant [52] would be the cause of the shorter delivery batch duration observed in autumn. Until now, negative seasonal effects in the pig industry included delayed puberty in gilts, prolonged weaning-to-estrus interval, reduced farrowing rate, and reduced litter size [53], but we did not find any reference to seasonal effects on delivery batch duration. On the other hand, under buserelin action, the delivery batch duration in spring was similar to that of autumn [49,54,55].

Seasonal effects on sperm characteristics may also play a role. Seasonal influence on protamine-like proteins has been demonstrate in invertebrates (*Myrtilus galloprovinciallis*); these proteins are the major basic component of sperm chromatin and several ambient factors (including pollutants) can modify their ability for binding and protecting DNA, so that their alteration allows sperm DNA fragmentation, causing infertility [56,57].

Several studies in mammals showed similar results. In humans, seasonal changes in sperm count and chromatin condensation were described with maximum values in January and April, respectively; no circannual relation was observed for motility and vitality [58]. However, circannual variation of morphology and motility of human semen were more recently reported; the rate of sperm with fast forward motility decreased from spring to autumn, with a recovery in winter, while the percentage of sperms with normal morphology was significantly higher in spring when compared with summer [59]. Seasonal effects on the chromatin status were also observed in semen from ovine, Iberian red deer and brown bear; in the breeding season, chromatin was less condensed and this status may be related to enhanced spermatogenesis [60]. In stallion semen, elevated DNA fragmentation and both low sperm motility and viability were detected in midsummer [61].

In boar, distinct motile sperm subpopulations occur in extended semen, and their proportions vary according to the season of collection; summer and autumn seem negative impact on the fast and linear subpopulation [62]. Additionally in boar, a reduction in sperm concentration was detected in spring and summer, although most seminal parameters were constant year-round [63]. As recently showed [64], DNA fragmentation was significantly higher when semen was produced in the increasing photoperiod respect to the decreasing photoperiod; the lowest values corresponded to autumn, while the highest values were found in summer.

Ambient pollutants can also play an important role in male fertility. In humans, metal pollutants as copper and chromium altered protamines/histones ratio and DNA binding model; therefore, the protective action of these proteins was reversed and they were involved in DNA oxidative damage, resulting in a higher DNA fragmentation index in the spermatozoa [65]. The effect of these metal pollutants could be transgenerational [66] Boar used in this study belonged to an insemination center, located in the countryside, and no industrial pollution was reported in its vicinity. Also, no nutritional problems affecting sperm production occurred in this center.

FTAI protocols including the use of GnRH analogues are aimed at improving piglets' weight at birth (individual and litter weight), as well as litter weight homogeneity [67]. Our protocol resulted in increased individual weight at birth, but no differences were found for litter weight and litter homogeneity. As can be seen in Table 5, mean piglet weight from C and T groups differed in only 0.023 kg (23 g). The sample sizes were large in both groups and the SE values were low; in these conditions, the power of statistical test is high and therefore very small differences may appear as statistically significant. A different issue is the biological significance of this very small difference detected as statistically significant; it only accounts for a 1.74% increase in weight. Furthermore, the resolution was 20 g for the used scale, very near the detected difference between means. The resolution

of a scale is the smallest readable difference between two measured values. On the other hand, delivery was not induced in this farm and no significant differences were detected between groups for gestation length; gestation length would not explain for the detected difference in birth weight. Hence, the applied hormonal treatment can be assumed not to worsen the newborn piglet's weight. No significant differences were found between groups for total piglets born/litter, and therefore, the slightly increased individual weight at birth in the T group was not reflected in the litter weight. Litter homogeneity was high in both the T and C groups; good management on the studied farm could explain this finding. Vangroenweghe [44] reported that treatment with another GnRH analogue (peforelin) did not result in a significant increase in individual weight at birth or litter weight homogeneity.

The proposed protocol implies the use of an additional treatment (buserelin) with respect to the usual protocol (double PCAI). However, since only one insemination is needed, the cost of semen, material, and personnel is reduced; therefore, the proposed protocol represents a 36% reduction in the cost of the usual protocol. Furthermore, the proposed protocol shows other advantages shown below [68]. Synchronizing ovulation and reducing the number of inseminations allow a better organization of farm work. Additionally, greater grouping of deliveries becomes possible, allowing better care of mothers and newborn piglets. Using a single dose of semen allows selected boars to be tested on a larger number of females in less time, so their progeny tests can be completed more quickly. In this way, a greater speed in the diffusion of genetic improvement is achieved in production farms. All these advantages are achieved without worsening the productive and reproductive indices, as shown in the present work.

5. Conclusions

The present study proposes, as a novelty, the combination in gilts of single FTAI plus buserelin with the PCAI technique. Only a small percentage of unsuccessful probe passage was found (10.2%; 23/226). The frequency of semen backflow, metritis, and bleeding was also small (global frequency: 14.8%; 30/203). There were no significant impacts on reproductive, prolificacy, and both gestation and weaning-AI interval duration parameters. Significant decreases in both estrus and delivery batch duration in spring were detected. Additionally, a significant increase in individual birth weight was found. According to our results, the use of the single FTAI-PCAI with buserelin is recommended in gilts. Using this new combined technique reduces both seminal doses and number of AIs; therefore, an important optimization of boars and semen doses is achieved. In this way, each gilt only needs 1500×10^6 spermatozoa (one dose) to get pregnant, versus the 6000×10^6 spermatozoa (two doses) needed with the usual technique.

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Data Availability Statement: Data supporting reported results can be sent to anyone interested by contacting the corresponding author.

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Discusión general y perspectivas de futuro

7

El uso de los actuales protocolos de inseminación artificial en las cerdas es consecuencia del desarrollo importante en la biotecnología reproductiva porcina que se han producido en las últimas décadas. La implementación de nuevas técnicas de inseminación (IAPC), así, como del uso de nuevas hormonas reproductivas para la inducción de la ovulación (buserelina) e inseminación única a tiempo fijo, se han incrementado a nivel mundial en los últimos años con el objetivo de mejorar la eficiencia reproductiva de las cerdas multíparas de las granjas porcinas comerciales (Bennenmann *et al.,* 2018), pero su aplicación en cerdas nulíparas es todavía muy incipiente. Así mismo, el fallo en la detección de la pubertad en las cerdas nulíparas de la granja se considera actualmente un serio problema, al observarse tras el sacrificio que muchas hembras eliminadas por anestro, eran púberes y podrían haber sido buenas hembras reproductoras.

Desde 1959, cuando Hancock (1959) describió y utilizó por primera vez la inseminación intrauterina no quirúrgica en cerdas multíparas, se han utilizado varios tipos de instrumentos, como endoscopios, catéteres o sondas y pipetas para desarrollar lo que hoy se conoce como IAPC. Existen ciertas limitaciones, incluida la falta de idoneidad para su aplicación en nulíparas Landrace x Large White (Hernández-Caravaca *et al.,* 2017; Llamas-López *et al.,* 2019).

La IAPC y la inducción de la ovulación en cerdas nulíparas son biotecnologías reproductivas innovadoras para mejorar la productividad porcina. Numerosos países como USA, Alemania o España han hecho esfuerzos, con más o menos éxito, para desarrollar nuevos protocolos de IAPC-IATF en las cerdas nulíparas (Brussow *et al.,* 2009; Knox *et al.,* 2017). Si los principales exportadores de carne de cerdo quieren ser competitivos en la producción porcina mundial, no solo deben ser capaces de producir grandes volúmenes de toneladas de carne, sino que deben tener una producción más eficiente y sostenible, utilizando nuevos protocolos reproductivos en sus granjas.

Por ello, es imperativo el desarrollo de nuevas biotecnologías. Para ello, es necesario conocer en profundidad los mejores métodos de diagnóstico de la

pubertad, de la técnica de IAPC y la inducción de la ovulación e IATF en cerdas nulíparas.

Esta Tesis Doctoral permite conocer en las cerdas nulíparas cómo el uso combinado de estas nuevas técnicas reproductivas puede mejorar o mantener los resultados de los parámetros reproductivos con los beneficios que proporcionan cada una de ellas. Nuestra investigación ha sido posible gracias al estudio de los diferentes métodos de diagnóstico de la pubertad, de la técnica de IAPC y de la aplicación de la buserelina para la inducción de la ovulación en cerdas nulíparas, dando como resultado tres publicaciones científicas y un artículo de revisión bibliográfica.

Hablar de la eficiencia reproductiva en las cerdas nulíparas es un tema importante que influye en los parámetros productivos futuros de la granja. El inicio de la pubertad es un proceso fisiológico complejo donde se asocian factores endocrinos y físicos. Aunque la edad de inicio de la pubertad está controlada en parte por la genética (moderadamente hereditaria, r=0,38) (Zack *et al.,* 2017; Malopolska *et al.,* 2018), también los factores externos y el manejo de las cerditas en la propia granja condicionan el momento de aparición del primer celo.

La frecuencia de cerdas eliminadas en las granjas porcinas es mayor en el grupo de las hembras nulíparas (38,5 a 51,1 %) (Engblom et al., 2007 y 2016). Alrededor del 10 % de las nulíparas son sacrificadas antes de su primera cubrición (Bolout, 2004), lo que provoca un grave impacto económico (Li et al., 2021; Stančič et al., 2021). La principal razón de eliminación de las cerdas nulíparas del ciclo productivo de una granja es la ausencia de signos externos de celo en las hembras mayores de meses (Falceto, 2016). Sin embargo, Stančič et al (2011) indican que los celos silentes (ovulación sin signos externos de manifestación del celo) ocurren solo en el 4 al 5 % de las cerdas nulíparas.

Además, se ha comprobado que muchas de las cerdas nulíparas sacrificadas (40-60 %) presentan aparatos genitales púberes (Falceto, 2016), por lo que un diagnóstico efectivo en granja podría evitar eliminar cerdas potencialmente buenas reproductoras cuyos celos no habían sido identificados correctamente

(Roongsitthichai *et al.,* 2013; Tani *et al.,* 2016). Un retraso en el diagnóstico de la pubertad conlleva también un aumento en la edad a la primera cubrición (220-300 días) que se ha asociado con un incremento en la granja del riesgo de descarte debido a los fallos en la fertilidad en un 2,1 % (Tani *et al.,* 2016 y Patterson y Foxcroft, 2019).

Los resultados del trabajo de investigación del **Artículo 1** demostraron que, en las cerdas nulíparas impúberes, los folículos ováricos parecen ser reclutados en oleadas, pero solo crecen hasta 6 mm de tamaño antes de sufrir atresia (Falceto, 2016, Knox, 2019). Mientras que los folículos mayores de 6 mm sólo aparecen en las cerdas jóvenes que han alcanzado la pubertad. La presencia de cuerpos lúteos y *albicans* es compatible con la existencia previa de uno o dos celos normales (Falceto, 2016).

Por otro lado, las cerdas nulíparas prepuberales (PRE) y pospuberales (PUB) diferían en el desarrollo de las diferentes secciones del tracto genital y de las secciones uterinas ($p < 0,001$) coincidiendo con los estudios de Kapelanski *et al* (2012), Žarković (2014), Oberlender *et al* (2014) y Graves *et al* (2019). Además, tal como indican Kauffold *et al* (2008), el aumento del desarrollo folicular se ha relacionado con el aumento del tamaño de todas las secciones del útero.

En nuestro trabajo de investigación también se comparó la capacidad de diferentes procedimientos de diagnóstico para detectar la pubertad en cerdas jóvenes en condiciones de granja, tales como: características externas de la cerda, medición del espesor de la grasa dorsal mediante ecografía y medición de la longitud de vagina-cérvix mediante un catéter de inseminación graduado, el análisis de la progesterona en sangre y la ecografía abdominal para evaluación ovárica y uterina. La evaluación se basó en parámetros de precisión (R2 de Nagelkerke, sensibilidad, especificidad, valor predictivo positivo, valor predictivo negativo y área bajo la curva ROC). Estos parámetros pueden utilizarse como orientación para elegir el mejor procedimiento de diagnóstico de la pubertad en las cerdas nulíparas de la granja.

Se ha descrito que la aparición espontánea del primer celo dentro de un lote de nulíparas se puede extender hasta 15 semanas entre la primera y la última cerda

(Després *et al.,* 1992; Martinat-Botté *et al.,* 2003). El momento de la pubertad está influenciado por la raza, el peso corporal, la grasa dorsal, la nutrición y la exposición al verraco (Evans y O'Doherty, 2001). La liberación de las gonadotropinas y la maduración de los folículos van a depender de estos factores (Foxcroft *et al.,* 2001; Tummaruk *et al.,* 2001; Amaral *et al.,* 2009).

Así, se ha observado, que las cerdas con una tasa de crecimiento alta alcanzan la pubertad antes que aquellas con una tasa menor (Young *et al.,* 2008; Tummaruk *et al.,* 2009). En condiciones de granja, la evaluación de la condición corporal se basa habitualmente en una clasificación mediante el examen visual de la cerda con puntuaciones que van del 1 al 5. Dado que esta observación depende de las habilidades individuales de puntuación del operario, se considera un método impreciso y subjetivo (Roongsitthichai y Tummaruk, 2014). Sin embargo, la medición de la grasa dorsal mediante un ecógrafo puede ser una técnica más objetiva para valorar la condición corporal. Por ello hemos elegido este parámetro como una herramienta predictiva del momento de la pubertad, ya que podría tener un impacto importante en la pubertad de las cerdas

Los factores internos (raza, peso corporal, grasa dorsal) intervienen en el inicio de la pubertad en las cerdas nulíparas, que están relacionados con el eje endocrino-reproductivo (Evans y Doherty, 2001). El peso corporal y la grasa dorsal tienen un impacto en la reproducción de las nulíparas en la liberación de gonadotropinas y en la maduración de los folículos (Foxcroft *et al.,* 2001). Los resultados del artículo 1, muestran diferencias significativas ($P < 0,001$) entre el espesor de la grasa dorsal de las cerdas nulíparas prepuberales (6,46 ± 1,93 mm) y las puberales (9,50 ± 2,469 mm) coincidiendo con Magnabosco *et al* (2016) y Rydhmer *et al* (1994) en que las hembras de crecimiento lento muestran menor grasa dorsal y una pubertad retrasada, siendo lo más probable que sean descartadas de la granja (Magnabosco *et al.,* 2016).

Otro método de diagnóstico de la pubertad que fue contrastado fue la determinación de la concentración de P4 en suero sanguíneo, tal como propone

Eliasson (1991). Esta hormona solo aumenta después de la pubertad (Esbenshade *et al.,* 1982; Evans y O'Doherty, 2001). Por lo tanto, las cerdas nulíparas que muestren una concentración de P4 mayor de 2 ng/ml serán consideradas púberes, pero por debajo de este valor se clasificarán como prepuberales (Martinat-Botté *et al.,* 2003; Kauffold *et al.,* 2004). Tal como cabría esperar, los resultados del artículo 1, muestran diferencias significativas ($P < 0,001$) entre los niveles de progesterona de las cerdas nulíparas prepuberales (0,84 ± 0,41 ng/ml) y las puberales (29,10 ± 27,94 ng/ml).

El aparato genital requiere la sucesión de estrógenos y progesterona de cada ciclo sexual para alcanzar el desarrollo que tiene la hembra adulta. En el animal vivo no se puede medir la capacidad uterina que alcanza el útero tras el primer celo. Sin embargo, Martin Rillo *et al* (2001) indico que la capacidad uterina está relacionada con la longitud de vagina-cérvix y por tanto su medición puede predecir la capacidad uterina de la cerda púber. Dado que es un parámetro fácil de medir lo hemos elegido herramienta de diagnóstico de la pubertad en nuestro trabajo de investigación. Nuestros resultados han mostrado diferencias significativas ($p = 0,003$) entre la longitud vagina-cérvix de las cerdas nulíparas prepuberales (21,75 ± 3,84 cm) y las puberales (27,35 ± 5,43 cm).

Por otro lado, la técnica de ultrasonografía abdominal ha sido recomendada por diferentes autores (Martinat-Botté *et al.,* 2003; Kauffold *et al.,* 2004) como un método preciso y menos laborioso para el diagnóstico de la pubertad en cerdas nulíparas en granjas comerciales, observando una precisión del 95 al 100 % cuando se examinan tanto el útero como los ovarios.

La ecografía puede evaluar a los ovarios y sus estructuras (Kauffold *et al.,* 2008). Se observa en nuestro trabajo qué los resultados más precisos se obtuvieron con un técnico cualificado en el uso de la ecografía reproductiva porcina independiente del ecógrafo utilizado, aunque, si se utilizan equipos con menor precisión se necesitará complementar el diagnostico con la combinación de otros métodos como la medición de la longitud de vagina- cérvix y la medición de la P4 (diferencias significativas al comparar los grupos PRE y PUB) para tener una mayor

precisión en la identificación de la pubertad en cerdas nulíparas.

Sin embargo, a nivel práctico en la granja, se deberán considerar factores adicionales (facilidad de uso, el coste y el efecto estresante en los animales) en la elección del procedimiento de diagnóstico de la pubertad, debido a que puede ser un desafío en algunas granjas la inversión necesaria en la compra de equipos y en la capacitación técnica básica para su utilización, mientras que si el equipo de ultrasonografía esta ya presente en la granja, su uso es más económico que el gasto del análisis de la concentración de progesterona. Además, causa menos molestias que la medición de la longitud de vagina-cérvix y toma menos tiempo si lo usa un técnico experimentado.

En el **Artículo 2** de la comparación de las técnicas de IAPC e IAC, se analizaron la efectividad de la aplicación de la sonda específica de IAPC utilizada para cerdas nulíparas y sus resultados productivos a nivel de granjas comerciales.

Sin duda, uno de los pilares de la producción porcina es la inseminación artificial. Esta técnica ha permitido que el sector porcino alcance los niveles actuales de producción cárnica mundial, además de facilitar el trabajo en las granjas, el avance de la mejora genética de los reproductores y reducir la transmisión de EV (Knox *et al.,* 2017; Martínez *et al.,* 2019; Falceto *et al.,* 2021).

Actualmente la IA es utilizada en más del 90 % de las granjas alrededor del mundo (Knox, 2016). En los últimos años se han desarrollado nuevas biotecnologías relacionadas con la optimización de la técnica de inseminación, el manejo del estro y la ovulación de la cerda (Falceto, 2018). Varios fueron los factores que ayudaron a la implementación de la técnica de la IA, entre ellos están: la especialización del sector porcino, las innovaciones en los materiales, los programas de mejoramiento genético y la eficiencia de los CIA en la producción de dosis seminales (Fitzgerald *et al.,* 2008; Wilson, 2012; Knox, 2016).

El uso de la inseminación artificial poscervical representa un aspecto importante de los avances en biotecnología reproductiva que se han producido en las explotaciones porcinas de todo el mundo en las últimas décadas. Sin embargo, tal

como se ha descrito en la introducción de la Tesis Doctoral, su aplicación se limita principalmente a las cerdas multíparas, al considerase poco idónea para su aplicación en cerdas nulíparas Landrace × Large White (Hernández-Caravaca *et al.,* 2017; Llamas-López *et al.,* 2019).

En este segundo experimento se comparan los datos de resultados de monta natural e IAC de Wilson (2012) con nuestros resultados IAPC en nulíparas, observando diferencias en el volumen y concentración de las dosis seminales, así como en el lugar de depósito de la dosis y el tiempo que tarda en realizarse cada técnica.

Las granjas del estudio diferían en varios factores importantes (año, número de IA/cerdas nulíparas, temporada, réplicas, primerizas/réplicas, tiempo de IA), aunque no hubo diferencias significativas ($p > 0,005$) de los resultados de fertilidad y prolificidad (total de lechones nacidos, nacidos vivos y nacidos muertos y momias/camada) entre los grupos de IAC y IAPC, coincidiendo con los resultados de Sbardella *et al* (2014).

Los resultados que hemos obtenido no apoyan la recomendación de Levis *et al* (2001) y Dallanora *et al* (2004) de no usar PCAI para cerdas jóvenes. Sin embargo, son coincidentes con los resultados de Sonderman (2016) y Ternus *et al* (2017), donde se concluyó que IAPC puede ser usado en cerdas jóvenes Landrace × Large White sin comprometer la eficiencia de producción porcina.

Con respecto al porcentaje de éxito de paso de sonda IAPC, en el presente estudio se observa relativamente alto (85,4 %) en comparación con los porcentajes que generalmente esta descrito por otros autores que han comparado cerdas nulíparas (< 85 %) con multíparas (95 %) (Sbardella et al., 2014; Bortolozzo et al., 2015; Hernández-Caravaca et al., 2017).

Otro resultado a destacar es que la frecuencia del reflujo de semen fue menor en el grupo IAPC comparado con el grupo IAC ($p = 0,006$). Rozeboom *et al* (2014) y Llamas-López *et al* (2019) desaconsejaron el uso de PCAI en cerdas jóvenes Landrace

× Large White, debido a que podrían causar lesiones en el cuello uterino. Sin embargo, los resultados del presente estudio indican un número muy pequeño de casos de sangrado (11/958: 1,1 % por IAPC) no detectándose diferencias significativas entre los grupos CAI y PCAI (P = 0,224).

En los últimos años, se han desarrollado nuevos catéteres de IA para evitar lesiones en las cerdas jóvenes y simplificar el uso del procedimiento de IAPC en granjas comerciales (García-Vázquez *et al.,* 2019). El uso una sonda de IAPC específica para cerdas jóvenes por parte de un técnico cualificado, podría explicar las diferencias de los resultados productivos de la Tesis Doctoral con las experiencias de otros autores. Poder usar únicamente la técnica de IAPC en todas las cerdas de una granja (nulíparas y multíparas) permite que todas las dosis seminales recibidas del centro de inseminación sean iguales con bajo volumen y numero de espermatozoides, simplificando la logística de trabajo en un lote de cubriciones.

Tal y como se describe en la revisión bibliográfica llevada a cabo en el **Articulo 3,** hasta el descubrimiento de los agonistas de la GnRH para la estimulación del crecimiento folicular y la inducción de la ovulación sólo eran usadas las gonadotropinas (Brüssow *et al.,* 1996). Los resultados de la bibliografía consultada demuestran la eficacia del uso de la eCG para sincronizar el desarrollo folicular y el uso de la pLH o hCG para la inducción de la ovulación en cerdas nulíparas (Ulguim *et al.,* 2014; Knox *et al.,* 2017). El uso de un agonista de la GnRH, la peforelina no mostró diferencias significativas en los parámetros reproductivos, aunque parecia tener un efecto positivo sobre crecimiento del folículo, pudiendo tener una cierta aplicación para nuevos protocolos de IATF (Brussow *et al.,* 2009; De Jong *et al.,* 2013; Vangroenweghe *et al.,* 2016).

Otros agonistas utilizados en los últimos años para la reproducción porcina son: gonadorelina, licerelina, peforelina, buserelina y triptorelina. Los resultados pueden variar debido a factores del desarrollo del folículo relacionados con los protocolos de inducción, el tipo de agonista, las dosis utilizadas y el momento de la administración (Knox, 2015; Pearodwong *et al.,* 2019).

En la actualidad, dentro de los agonistas de la GnRH, además del uso de la

buserelina intramuscular, se está promoviendo la aplicación intravaginal de un gel que contiene un agonista de la GnRH (triptorelina) para cerdas multíparas y nulíparas (Baer y Bilkei, 2004; Stewart *et al.,* 2014; Kirkwood y Kauffold, 2015; Knox *et al.,* 2017). Aunque, en los países donde se comercializa sólo se recomienda el uso de triptorelina en cerdas multíparas destetadas. El uso de 100–400 µg de triptorelina con IATF en cerdas nulíparas 120 h después del tratamiento con altrenogest obtuvo excelentes resultados para la inducción de la ovulación, pero es posible que aún no se haya identificado el momento óptimo de administración de triptorelina después del protocolo de sincronización del celo (Knox *et al.,* 2017). Por el contrario, en otro estudio, un único protocolo de triptorelina IATF-IAC aplicado 120 h después del tratamiento con Altrenogest en cerdas nulíparas mostró una tasa de parto más baja que en el grupo de control (80,3 % frente a 89,5 %) (Rodrigues *et al.,* 2020). Se recomienda realizar más investigaciones sobre este y otros agonistas de la GnRH para desarrollar nuevos protocolos de IATF en cerdas nulíparas. Actualmente, en Europa solo esta comercializada la buserelina para uso en las cerdas nulíparas, por lo que es el agonista que hemos elegido en esta esta Tesis Doctoral.

Por último, en el **Artículo 4**, hasta donde se sabe, no se había realizado previamente ningún trabajo de investigación que combine el uso de la IAPC con una dosis seminal única a tiempo fijo en cerdas nulíparas tras la inducción de la ovulación con buserelina. Los resultados de este trabajo demostraron de nuevo el éxito del paso de la sonda IAPC para cerdas nulíparas (> 85 %) afirmando los resultados del **Artículo 2.** La frecuencia de problemas durante la IA (reflujo de semen, metritis y sangrado) no presentó diferencias significativas entre los grupos. Estos resultados demuestran otra vez que el uso de un catéter específico para IAPC en cerdas nulíparas por parte de un técnico calificado se recomienda para la implementación de nuevos protocolos de IA (Ausejo *et al.,* 2018).

El procedimiento realizado en la última experiencia utiliza la IAPC a tiempo fijo tras la inducción hormonal de la ovulación con buserelina, con una única dosis seminal/cerda nulípara en celo. Además del uso eficiente de las dosis seminales en la granja, otra ventaja obtenida ha sido que los partos han estado más sincronizados

en todo el lote de cerdas nulíparas inseminadas, al igual que ha ocurrido en otras investigaciones realizadas en las cerdas multíparas (Falceto *et al.,* 2014).

De esta forma puede obtenerse una mayor eficiencia del trabajo en la sala de maternidad debido a una mejor supervisión del parto, encalostramiento, adopciones y un procesamiento eficiente de las camadas (Ulguim *et al.,* 2014; Rodrigues *et al.,* 2020).

Por lo general, las técnicas descritas de IATF que incluyen el uso de agonistas de la GnRH tienen como objetivo mejorar el peso de los lechones al nacer (peso individual y de la camada), así como la homogeneidad del peso de la camada (McBride, 2019). Nuestro protocolo dio como resultado un aumento del peso individual en el nacimiento, pero no se encontraron diferencias significativas en el peso ni en la homogeneidad de la camada coincidiendo con los resultados de Vangroenweghe et al. (2016) tras el uso de otro agonista de la GnRH (peforelina).

Se ha abordado también la estacionalidad reproductiva de la cerda. Antes del otoño es frecuente una bajada de los todos los parámetros reproductivos. Este síndrome es una reminiscencia heredara de su antepasado silvestre, el jabalí (*Sus scrofa*), especie considerada como reproductora de días cortos en la que los factores externos (temperatura, luz y alimentación) controlan el funcionamiento del eje HHO y seleccionan el otoño como la mejor época del año para la reproducción (Mauget, 1982).

Los efectos estacionales negativos en la industria porcina incluyen entre otros aspectos, el retraso de la pubertad y un mayor porcentaje de cerdas nulíparas en anestro (Peltoniemi *et al.,* 2000), acompañado de una reducción de la tasa de parto y un tamaño reducido de la camada en las cerdas primerizas inseminadas (Tast *et al.,* 2001). Sin embargo, en el trabajo de la Tesis Doctoral, bajo la acción de la buserelina como inductora de la ovulación en las cerdas nulíparas, los resultados de los parámetros reproductivos en primavera fueron similares a los del otoño, mejorándose los resultados productivos esperados según los datos de otros autores (Ziecik *et al.,* 1983; Soede *et al.,* 2011).

El protocolo usado en esta experiencia para la IATF-IAPC, implica el uso de un tratamiento de buserelina con una sola dosis seminal respecto al protocolo habitual realizado en las granjas (doble IAPC). Como solo se necesita una IA, se minimiza el coste del semen, material y personal; por tanto, esta forma de trabajo supone una reducción del 36 % del coste económico frente al procedimiento convencional. Además, muestra otras ventajas que se exponen a continuación (Falceto, 2018), sin empeorar los índices productivos y reproductivos:

- La sincronización de la ovulación y la reducción del número de inseminaciones permiten una mejor organización del trabajo de la granja.
- Se hace posible una mayor agrupación de partos, lo que permite una mejor atención de las madres y los lechones recién nacidos.
- El uso de una sola dosis de semen permite que los verracos seleccionados genéticamente se analicen en un mayor número de hembras en menos tiempo, por lo que sus pruebas de progenie se pueden completar más rápidamente. De esta forma se consigue una mayor velocidad en la difusión de la mejora genética en las explotaciones de producción.

Actualmente tanto la sociedad como el sector porcino están demandando el desarrollo de nuevas tecnologías en equipos y protocolos de IA que al mismo tiempo que mejoren los índices productivos sean más sostenibles ya que se reducen el número de catéteres utilizados a uno por celo y cerda.

Los resultados presentados en esta Tesis Doctoral sientan las bases para continuar con otros temas de estudio de los avances en nuevas biotecnologías reproductivas porcinas en cerdas nulíparas que ya se están estudiando en las hembras multíparas como el uso de semen sexado o congelado. Incluso la inseminación artificial única con espermatozoides encapsulados que garantiza que haya espermatozoides en el oviducto en el momento de la ovulación (Sánchez-Sánchez *et al., 2*022).

Conclusiones 8

1. La ecografía como método de diagnóstico de la pubertad en cerdas nulíparas proporcionó mejores resultados que otras técnicas diagnósticas. Estos resultados resaltan la necesidad de técnicos experimentados en el uso de la ecografía reproductiva a nivel de granjas comerciales.

2. La medición de la longitud de la vagina y el cérvix de la cerda nulípara con un catéter graduado combinado con la determinación de progesterona sanguínea podría ser un protocolo eficaz en la granja cuando no diferencien las hembras en anestro prepuberal de las púberes mediante el uso de la ultrasonografía.

3. La inseminación artificial poscervical mediante un catéter específico para cerdas nulíparas, permite resultados reproductivos similares a los observados con la inseminación cervical, pero con las ventajas de la técnica poscervical. Siendo insignificantes las complicaciones, pese al incompleto desarrollo del aparato genital.

4. El protocolo reproductivo de la inseminación artificial a tiempo fijo con una sola dosis seminal por cerda nulípara en celo, utilizando la inducción de la ovulación con buserelina y la técnica poscervical ya comentada, presenta resultados reproductivos similares al uso de dos o más inseminaciones por celo realizadas sin inducción de la ovulación.

5. Todos los avances en la biotecnología reproductiva usados habitualmente en las cerdas multíparas han resultado ser eficaces en las cerdas nulíparas. En un futuro próximo se podrá utilizar la inseminación artificial poscervical en la mayoría de las cerdas nulíparas, además de poder inducir la ovulación para realizar una sola inseminación por cerda en celo e incluso utilizar semen congelado, encapsulado o sexado nivel de granja comercial.

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Apéndice 10

10.1 Características de las revistas científicas

Tabla 2. Factor de impacto y áreas temáticas de las revistas

REVISTA	FACTOR DE IMPACTO (JCR)	QUARTIL	ÁREA TEMÁTICA
Porcine Health Management	3,535	Q1(12/144)	VETERINARY SCIENCES
Animal Reproduction Science	1,660	Q2 (22/63)	AGRICULTURE, DAIRY & ANIMAL SCIENCE
Animals	3,231	Q1(16/144)	VETERINARY SCIENCES
Reproduction in Domestic Animals	1,858	Q2(55/145)	VETERINARY SCIENCES

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