UNIVERSIDAD DE LEÓN

Facultad de Veterinaria
Departamento de Sanidad Animal

TESIS DOCTORAL

Nuevas estrategias en el manejo hormonal de la reproducción en las cerdas nulíparas

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2013

AUTORIZACION DE LOS DIRECTORES DE TESIS PARA SU PRESENTACION

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La defensa de la Tesis Doctoral titulada: ¨**Nuevas estrategias en el manejo hormonal de la reproducción en las cerdas nulíparas**¨, dentro del programa de doctorado Sanidad Animal y Reproducción.

Y para dar cumplimiento al Art. 8º. 1 del R.D. 778/98, se firma en León a 20 de Noviembre de 2013,

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CONFORMIDAD DEL DEPARTAMENTO

El Departamento de Sanidad Animal, ha acordado dar su conformidad a la admisión a trámite de la lectura de la Tesis Doctoral titulada: ¨**NUEVAS ESTRATEGIAS EN EL MANEJO HORMONAL DE LA REPRODUCCION EN LAS CERDAS NULIPARAS**¨, dirigida por los Dres. **D. JUAN CARLOS DOMINGUEZ FERNANDEZ DE TEJERINA** y Dña. **BEATRIZ ALEGRE GUTIERREZ**, y elaborada por el tesando **D. RODRIGO MANJARIN ARREDONDO**.

Lo que se firma, para dar cumplimiento del Art. 8º. 2 del R.D. 778/98, en León a 20 de Noviembre de 2013,

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El director del Departamento El secretario del Departamento

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Esta memoria ha sido realizada en las instalaciones de las Facultades de Veterinaria de la Universidad Estatal de Michigan, USA, la Universidad de Guelph, Canadá, y la Universidad de León, asi como el Servicio de Técnicas Instrumentales de la Universidad de León, e instalaciones ganaderas de GPA en Ávila y UVESA en León.

Los resultados de esta memoria han sido presentados en los siguientes congresos:

American Society Animal Science 2010 Midwest Meeting

American Society Animal Science 2009 Midwest Meeting

Animal Science Graduate Research Forum, Michigan State University, September 2009

Animal Science Graduate Research Forum, Michigan State University, September 2008

A mi familia, mis directores de tesis y mis amigos por confiar en mí y darme su apoyo incondicional durante este periodo, gracias.

Agradecimientos

- En estas breves líneas, me gustaría agradecer a todas las personas e instituciones que han colaborado en la realización de este proyecto.
- A mis directores de Tesis, por darme la oportunidad de realizar estos experimentos y por su continuado esfuerzo y apoyo durante todo este periodo
- Dr. Roy Kirkwood, Dr. Robert Friendship and Dr. Glen Cassar, for their continued support, advice and education during my studies in the USA and Canada
- M.J. Castro y Evaristo Aguado, porque sin su inmensa ayuda nunca habría sido posible realizar esta investigación.
- Blas, Julio, Javier, Fernando, Víctor, Chechu, Noemí, Blanca y Saúl, que contribuyeron a esta tesis con su tiempo y comprensión.
- A Intervet International, por financiar parte de esta investigación.

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Índice de Abreviaciones

AI = inseminación artificial

Dpc = días post coito

eCG = gonadotropina coriónica equina

FSH = hormona folículo estimulante

GnRH = hormona liberadora de gonadotropinas

hCG = gonadotropina coriónica humana

IM = intramuscular

IU = unidades internacionales

LH = hormona luteinizante

NPD = días no productivos

PVP = polyvinylpyrrolidinone

SC = subcutáneo

SEM = error estándar de la media

IGF-1= factor de crecimiento similar a la insulina tipo I

IGF BP = proteína de unión al factor de crecimiento similar a la insulina tipo I

PAPP-A = Proteína plasmática A asociada a la preñez

CAPÍTULO 1: Introducción y objetivos

1.1 Introducción

La producción de una explotación porcina se mide por el núero de lechones destetados por semana, parámetro que depende fundamentalmente del porcentaje de hembras inseminadas semanalmente (Dial et al., 1996). La habilidad de alcanzar y/o mantener un adecuad número de inseminaciones semanal requiere un suministro continuo de cerdas nulíparas en celo. Esto es posible mediante la estimulación temprana de la pubertad en las hembras de reemplazo, seguida del mantenimiento de una actividad cíclica regular en las semanas previas a la fecha de inseminación (Kirkwood, 1999). Los métodos más utilizados para la estimulación temprana de la pubertad en porcino son la exposición de las hembras nulíparas a un macho adulto y la administración de hormonas gonadotrópicas. La adecuada exposición al verraco para estimular la pubertad y mantener ciclos estrales regulares hasta la primera inseminación, requiere el contacto directo diario del cerdo con las hembras nulíparas durante varios meses (Kirkwood and Thacker, 1992; Hughes, 1997). Cuando esto no es físicamente posible, o cuando la exposición diaria no revierte en la maduración sexual o la ciclicidad regular de las hembras, se vuelve necesaria la inducción del celo mediante un protocolo hormonal. La mayoría de preparaciones comerciales para la estimulación del celo en ganado porcino contienen una combinación de las hormonas gonadotropina coriónica equina (eCG) y gonadotropina coriónica humana (hCG), que presentan una acción folículo estimulante y luteinizante similar a las hormonas FSH y LH sintetizadas endógenamente en la hipófisis del cerdo. La preparación más común de estas dos hormonas está compuesta por 400 IU de eCG y 200 IU de hCG (PG600, Intervet, Schering-Plough Animal Health), y es altamente efectiva en la inducción del celo en cerdas destetadas; sin embargo, cuando es aplicada a cerdas prepúberes, hasta el 30% pueden llegar a no mostrar celos, y cerca del 30% de aquellos que si ovulan no desarrollan ciclos regulares en las semanas o meses posteriores

al tratamiento (Kirkwood, 1999). La etiología de la impredictibilidad de la respuesta estral y ovulatoria en cerdas nulíparas a la inyección de PG600 es desconocida.

La principal hipótesis de este trabajo es que la falta de respuesta estral en el 30% de cerdas prepúberes tratadas con hormonas exógenas es debida a una inadecuada combinación de las gonadotropinas eCG y hCG para promover el correcto crecimiento folicular y la instauración de la pubertad. Para demostrar esta hipótesis, 6 diferentes experimentos fueron llevados a cabo con el objetivo de comparar las respuestas estral y ovulatoria inducida por la administración de PG600 con los siguientes protocolos hormonales:

i. administración de eCG sola,

ii. pretratamiento con FSH seguido con eCG,

iii. administración de hCG sola,

iv. pretratamiento con FSH seguido con hCG,

v. tratamiento con eCG seguido de hCG,

vi. tratamiento con eCG + hCG seguido de hCG.

Los tratamientos *i* y *ii* están presentados en los capítulos 3 y 4 de la tesis, respectivamente, los tratamientos *iii* y *iv* en el capítulo 5, y los protocolos *v* y *vi* se recogen en los capítulos 6, 7 y 8. Finalmente, desarrollamos un último experimento presentado en forma de anexo, donde la fertilidad en respuesta a los diferentes protocolos hormonales fue evaluada en cerdas en anestro estacional, que representan un modelo fisiológico similar a las cerdas prepúberes.

Previos experimentos han demostrado que la mayoría de las cerdas prepúberes tratadas con PG600 que no desarrollan celo, presentan niveles posovulatorios de progesterona (P4) en sangre 10 días (d) después del tratamiento (Tilton et al., 1995). Estos resultados nos llevaron a especular de que la gonadotropina hCG, siendo un análogo de la hormona luteinizante (LH), hubiera podido causar la ovulación prematura o incluso la luteinización de los folículos más grandes, de forma que el aumento adelantado de P4 bloquearía el comportamiento estral en las cerdas prepúberes. Si esta hipótesis es cierta, entonces la inyección sola de eCG resultaría en una mejoría de la respuesta estral, puesto que esta hormona tiene muy poca actividad LH en comparación con la hCG. En apoyo de esta hipótesis, la inyección de dosis elevadas de eCG sin el componente hCG indujo la ovulación del 70-100% de las cerdas prepúberes tratadas (Dial et al., 1984; Britt et al., 1986; Esbenshade, 1987; Flowers et al., 1989; Bolamba, 1992). Sin embargo, la comparación de la respuesta estral y ovulatoria entre la administración de eCG y la combinación de eCG y hCG nunca ha sido evaluada en cerdas prepúberes. Además, es desconocida si la diferencia en respuesta estral y ovulatoria entre ambos protocolos hormonales está relacionada con el desarrollo folicular y el peso de las hembras al momento del tratamiento. Por lo tanto, el primer objetivo de la tesis consistió en determinar el efecto de la administración de eCG sola o en combinación con hCG sobre el celo y la ovulación de cerdas prepúberes. Además, la relación entre estas respuestas, el peso y el desarrollo folicular inicial de las hembras, es también discutida. El celo en las hembras prepúberes fue valorado mediante la expresión del reflejo de inmovilidad inducido por la exposición directa a un macho durante 15 min entre los 2 y 7 d posteriores a la inyección de las gonadotropinas. Por el contrario, la ovulación fue cuantificada mediante el análisis de la concentración de P4 en muestras de sangre obtenidas de la vena yugular en los d 0, 3 y 10 respecto a la inyección de las gonadotropinas. Una elevación en la concentración de P4 $\geq$ 1 ng/mL entre los d 0 y 3 postratamiento fue definida

como ovulación prematura, mientras que niveles sanguíneos de P4 < 1 ng/mL en los d 0 y 3, seguidos de un aumento en la concentración de P4 ≥ 5 ng/mL en el d 10 fue definido como una respuesta ovulatoria normal. El crecimiento folicular previo a la inyección de gonadotropinas fue determinado por ultrasonografía ovárica mediante la utilización de una sonda transrectal, que permitió la valoración del diámetro de los 3 folículos mas grandes presentes en ambos ovarios.

El segundo objetivo de este trabajo consistió en determinar el efecto de la inyección consecutiva de las hormonas FSH y eCG en la respuesta estral y ovulatoria en cerdas nulíparas. Numerosos experimentos en cerdas prepúberes han mostrado la imprescindible función de la hormona folículo estimulante (FSH) en el reclutamiento de los folículos primarios y secundarios, asi como el papel de la hormona eCG en el desarrollo de folículos terciarios y preovulatorios (Guthrie et al., 1990; Knox and Zimmerman, 1993, Bolamba et al., 1996; Guthrie, 2005). Sin embargo, estas dos hormonas nunca han sido administradas de forma consecutiva en porcino para promover el desarrollo de la pubertad. Basándonos en estos experimentos, asi como en los resultados del primer experimento, formulamos la hipótesis de que, comparado con la administración eCG sola o en combinación con hCG, la administración previa de FSH seguida de eCG aumentaría las respuestas estral y ovulatoria en cerdas prepúberes. Debido a la corta vida media de la hormona FSH en sangre, esta fue administrada en 6 consecutivas inyecciones en intervalos de 12 h, seguido de una sola inyección de eCG 12 horas (h) después de la última administración de FSH.

El tercer y cuarto objetivos de este trabajo consistieron en determinar el efecto de la hormona hCG, administrada sola o de forma consecutiva a la inyección de FSH, en las

respuestas estral y ovulatoria en cerdas nulíparas. Previos experimentos han demostrado que la hormona hCG, además de su acciones ovulatoria y esteroidegenica, puede estimular también el crecimiento folicular (Guthrie et al., 1990; Bolamba et al., 1991; Driancourt et al., 1992). Sin embargo, en estos experimentos el diámetro y número de la población folicular al momento del tratamiento condicionó la capacidad de ovulación, sugiriendo la incapacidad de hCG para iniciar el desarrollo de los folículos más pequeños (Bolamba et al., 1991; Driancourt et al., 1995). Este efecto contrasta con la acción de la hormona FSH en el desarrollo de los folículos primarios y secundarios descrita previamente. Sin embargo, las hormonas FSH y hCG nunca han sido administradas de forma consecutiva en porcino para promover la ovulación. De esta forma, formulamos la hipótesis de que, comparado con la administración individual de hCG o en combinación con eCG, la administración de FSH seguido de hCG aumentaría la respuesta estral y ovulatoria en cerdas prepúberes. Con el objetivo de alargar la actividad biológica de la hormona FSH en sangre, esta hormona fue disuelta en una solución con polyvinylpyrrolidinone (PVP; Jackson et al., 2006) y administrada en dos inyecciones consecutivas separadas por 24 h, seguido de la administración de hCG 24 h después de la ultima administración de FSH.

El conjunto de los resultados obtenidos durante los experimentos anteriores nos llevó a formular la hipótesis de que la falta de respuesta estral y ovulatoria en 30% de las cerdas prepúberes tratadas con la PG600 podría ser debida a una insuficiente duración o intensidad de la actividad LH para completar el desarrollo folicular. Por lo tanto, la administración adicional de hCG, de forma concurrente o consecutiva a la administración de PG600, induciría un aumento en la tasa de celo y ovulación en cerdas prepúberes. Para probar esta hipótesis se llevaron a cabo dos diferentes experimentos (cuarto, quinto en la tesis). En el cuarto experimento recogido en la tesis, cerdas nulíparas de diferentes edades y pesos fueron

inyectadas con PG600, PG600 seguido de hCG, eCG seguido de hCG, o sirvieron como control. La suplementación con 200IU de hCG se realizo a las 0, 24 y 48h. La valoración de la respuesta ovulatoria se basó, al igual que en los casos anteriores, en un aumento en la concentración de P4 en sangre ≥ 5 ng/mL en el d 10. Además, se contabilizó el porcentaje de cerdas nulíparas con niveles de P4 ≥ 30 ng/mL, indicativo de una mejor respuesta ovulatoria debido a un mayor número de cuerpos lúteos, o a una mayor producción de P4 por cuerpo lúteo.

En base a los resultados obtenidos, se llevo a cabo el quinto experimento para investigar la etiología de los altos niveles de P4 asociados a la suplementación con hCG en las cerdas prepúberes. En este experimento se utilizaron 49 cerdas nulíparas que fueron tratadas con PG600 y dos dosis diferentes de hCG a las 24h. Veintiocho animales fueron sacrificados a los 10 d posteriores al tratamiento, y los ovarios fueron examinados para contabilizar el número de cuerpos lúteos y quistes foliculares, asi como la naturaleza de los mismos. El crecimiento folicular en el resto de los animales fue monitorizado usando el método ultrasonografía transrectal descrito anteriormente.

1.2 Objetivos

Objetivo general

Determinar la etiología de la impredictibilidad de las respuestas estral y ovulatoria en cerdas prepúberes a la inyección de gonadotropinas exógenas, y mejorar los protocolos hormonales actuales empleados en la inducción temprana de la pubertad en porcino.

Objetivos específicos

1. Valorar la eficacia de nuevos protocolos hormonales en la inducción del celo en cerdas prepúberes:
    a. eCG sola o consecutiva a la administración de FSH
    b. hCG sola o consecutiva a la administración de FSH
    c. eCG consecutiva a la administración de hCG
    d. hCG consecutiva a la administración de PG600 (eCG+hCG)

2. Determinar el efecto de la inyección de gonadotropinas exógenas en la respuesta ovulatoria en cerdas prepúberes:
    a. Niveles de progesterona en sangre
    b. Cuantificación de cuerpos lúteos
    c. Formación de quistes ováricos

3. Determinar la relación entre la madurez fisiológica de las cerdas prepúberes y las respuestas estral y ovulatoria a la administración de gonadotropinas exógenas:
    a. efecto del desarrollo folicular previo al tratamiento hormonal
    b. efecto del peso
    c. efecto de la edad

CAPÍTULO 2: Revisión Bibliográfica

2.1 Ovogénesis y desarrollo folicular en el cerdo

Durante el periodo embrionario temprano, comprendido entre los días 24 a 26 después del nacimiento, las células germinales primordiales migran desde el saco vitelino hasta la gónada indiferenciada, también llamada cresta genital, y se organizan en los cordones sexuales. Las células germinales llevan a cabo la primera división mitótica, aumentando su número desde 5×10^3 en el día 24 posfecundación (pfc) hasta más de 1×10^6 en el día 50. En ausencia del factor determinante testicular, los cordones sexuales se fragmentan en grupos celulares, cada uno de ellos rodeando a una de las células germinales. Estos grupos se diferencian en las células foliculares primitivas, y la mayor parte de la cresta genital se transforma en el ovario. Las células germinales se transforman en oogonias mediante sucesivas divisiones mitóticas y luego a oocitos primarios a través de la primera división meiótica. La división de los oocitos se detiene en la fase G_2 del ciclo celular, y no continúan la meiosis hasta inmediatamente después de la ovulación. El tiempo necesario para la transformación de oogonias a oocitos es más largo en cerdos que en otras especies de mamíferos (Black and Erickson, 1968). La primera oogonia entra en la profase meiótica antes del día 40 pfc y su transformación no es completa hasta aproximadamente 30 día después del parto **(Figura 2.1).**

Los oocitos primarios están rodeados por una o dos capas de células foliculares aplanadas que forman los folículos primordiales ováricos. Los primeros folículos primordiales son observados hacia el día 56 pfc, y su número aumenta a lo largo de la vida prenatal (Bielańska-Osuchowska, 2006). Aproximadamente 500.000 folículos primordiales están presentes en ambos ovarios en el día 10 después del nacimiento en la cerda, y

constituyen el número máximo disponible durante toda su vida reproductiva (Black and Erickson, 1968).

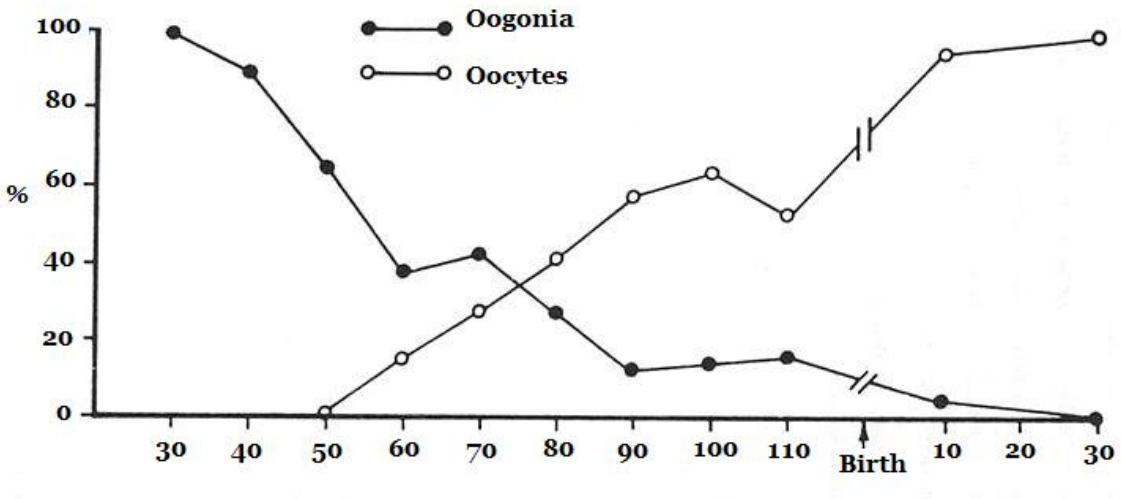

Figura 2.1 Distribución de las oogonias y oocitos durante el periodo embrionario y posterior al nacimiento en la cerda (tomado de Black and Erickson, 1968)

Dentro de los primeros 10 días después del nacimiento, algunos folículos primordiales se transforman en folículos primarios (0.12 mm), que consisten en oocitos con una a tres capas de células de la granulosa (Knox, 2000). La iniciación del crecimiento de los folículos primordiales implica tanto la acción hormonal a nivel sistémico como el efecto regulador de factores locales producidos por las células somáticas del folículo (Hirshfield, 1991) y, probablemente, de la propios oocitos en crecimiento (Picton et al., 1998). Las células foliculares continúan proliferando para formar varias capas de células de la granulosa, así como las células de la teca que las rodean, transformado los folículos primarios en secundarios (Sacristán et al., 1996; **Figura 2.2**). La transformación de folículo primario a secundario es independiente de las gonadotropinas hipofisarias, ya que se observan también

en los animales hipofisectomizados. El folículo entra en la etapa secundaria con 3 a 20 capas de células de la granulosa y un diámetro de 0.14 a 0.40 mm. El crecimiento más allá de los 0.4 mm se asocia con la formación del antro o folículo terciario. Los folículos antrales tienen una amplia variación en su diámetro (0.4-1.5 mm), asi como en el numero de capas de células de la granulosa (10-30; Knox 2005). El fluido folicular deriva tanto de la sangre, como de sustancias sintetizadas por la células de la granulosa y teca. Durante la fase antral, las células de la granulosa siguen proliferando y empiezan a desarrollar receptores para la hormona folículo estimulante (FSH). Al mismo tiempo, las células de la teca aparecen completamente diferenciadas formando dos capas concéntricas: la teca interna, con receptores para la hormona luteinizante, (LH) y la teca externa.

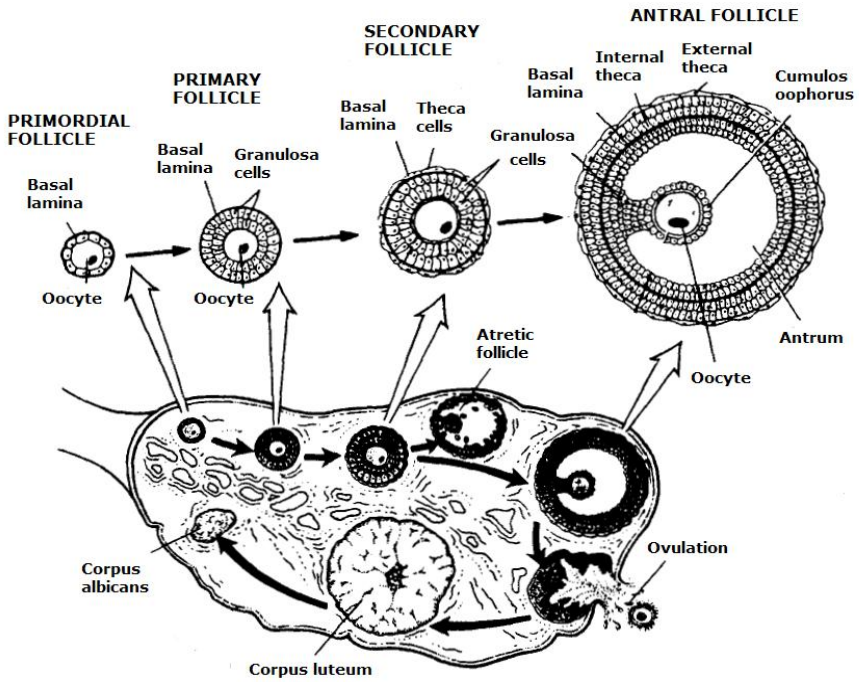

Figure 2.2 Figura representativa de la estructura del ovario y de las diferentes fases del desarrollo del folículo, cuerpo lúteo y cuerpo albicans (Tomado de Sacristán, 1996)

Durante la maduración folicular, las células de la teca y la granulosa sintetizan y secretan grandes cantidades de esteroides, que junto con las hormonas hipofisarias FSH y LH, permiten a los folículos crecer y alcanzar su desarrollo final (Cárdenas and Pope, 2002). El mecanismo implicado consiste en que, durante el desarrollo folicular, la LH se une a receptores de membrana LH-específicos localizados en las células de la teca interna del folículo en desarrollo, activando una cascada de eventos intracelulares resultantes en última instancia en la conversión de colesterol a testosterona. La testosterona se difunde fuera de las células de la teca interna y entra en las células de la granulosa, que tienen receptores para FSH. Cuando la FSH se une a su receptor, provoca la conversión de testosterona a estradiol mediante la enzima aromatasa. Al mismo tiempo, el estradiol, junto con el factor de crecimiento insulínico 1 (IGF-1), complementa el efecto de la FSH y LH para aumentar aún más la actividad de la aromatasa en los folículos.

Una vez que el folículo alcanza un tamaño crítico, las células de la granulosa unidas a la lámina basal se distancian del complejo oocito-cumulus, y establecen un segundo microambiente dentro el folículo (Richards, 2001). Esta separación requiere la regulación de las células de la granulosa por parte de factores y hormonas distintas a las del complejo oocito-cumulus. Localmente, la FSH induce cambios específicos en la expresión génica de las células de la granulosa, aumenta su proliferación, e induce la formación del antro que separa el componente oocito-cumulus de la capa lateral de células endocrinas. En última instancia, las células de la granulosa expresan también receptores para la LH, asi como la enzima P450 aromatasa , activina e inhibina, mientras que las células de la teca se diferencian para producir andrógenos a través de la ruta biosintética $P450_{17\alpha}$. Todos estos cambios aumentan aún más la esteroidogénesis en el ovario, inhiben la secreción hipofisaria de FSH, de forma que los folículos de menor diámetro, cuyo desarrollo depende de la FSH, sufren

atresia, y conduce a la retroalimentación positiva del estradiol en el hipotálamo para desencadenar el pico ovulatorio de LH.

2.2 Perfiles hormonales y desarrollo ovárico antes de la pubertad

2.2.1 Secreción de LH y FSH

En las cerdas prepúberes, la LH es una hormona clave en el control del desarrollo ovárico y la edad a la cual las cerdas alcanzan la pubertad (Evans and O'Doherty, 2001). Las concentraciones de LH en sangre disminuyen desde el nacimiento hasta alrededor del día 40 de edad, aumentan hasta los 120 días, y luego se reducen de nuevo hasta su punto más bajo cerca de los 180 días (Colenbrander et al., 1977; Pelletier et al., 1981; Diekman et al., 1983; Camous et al., 1985) (**Figura 2.3**). Desde los 180 días hasta el desencadenamiento de la primera ovulación, y por tanto el establecimiento de la pubertad, las concentraciones de LH en la cerda aumentan progresivamente (Pelletier et al., 1981; Prunier et al., 1993), al igual que en la mayoría de las especies. Este aumento en la secreción de LH cercano a la pubertad se caracteriza por un aumento en la media de la concentración de LH, asi como incremento en la frecuencia pulsátil (Pelletier et al., 1981; Prunier et al., 1993), y está asociada con la maduración final de los folículos ováricos (Beltranena et al., 1993), culminando en el pico de LH preovulatorio.

Las concentraciones de FSH en la sangre son altas entre el nacimiento y los 70-125 días de edad, y luego disminuyen hasta el establecimiento de la pubertad (Diekman et al., 1983; Camous et al., 1985; Prunier et al., 1993). Las concentraciones de estradiol son bajas durante la mayor parte del período prepuberal, y solo aumentan antes de la pubertad

(Esbenshade et al., 1982; Lutz et al., 1984; Camous et al., 1985). Las concentraciones de progesterona sólo aumentan después de la pubertad tras la formación de los primeros cuerpos lúteos (Esbenshade et al., 1982; Prunier et al., 1993).

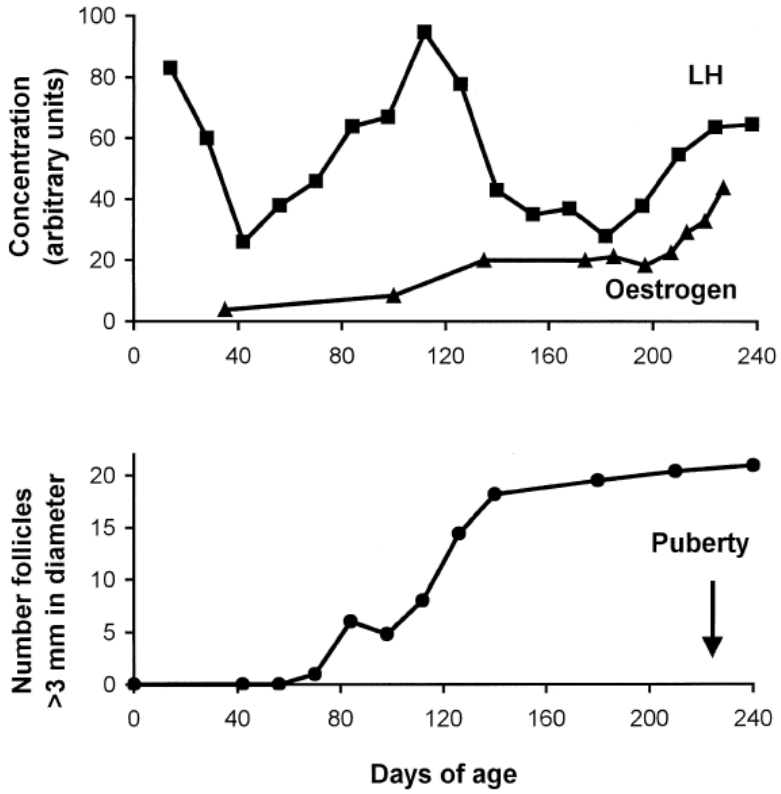

Figura 2.3 Relación entre la concentración media plasmática de estrógenos y LH, y el numero de folículos de diámetro mayor de 3 mm, durante la maduración sexual de la cerda (Tomado de Evans et al. 2001).

2.2.2 Regulación de la secreción de gonadotropinas (la teoría gonadostática)

La secreción de las hormonas hipofisarias FSH y LH durante el período prepuberal está regulada a través de la hormona liberadora de gonadotropinas (GnRH). La síntesis de GnRH en la hembra se lleva a cabo en las células neurosecretoras ubicadas en dos áreas

separadas en el hipotálamo, el centro tónico y el centro pulsátil. Anatómicamente, el centro pulsátil se compone de tres núcleos hipotalámicos conocidos como el núcleo preóptico, el área hipotalámica anterior y el núcleo supraquiasmático, mientras que el centro tónico consta solo de dos núcleos, el ventromedial y el arqueado. Los axones de las células de los centros tónico y pulsátil se extienden en las región del tallo hipofisario, donde los nervios terminan en una red capilar llamada sistema portal hipotálamo-hipofisario. Este sistema permite a la GnRH actuar inmediatamente sobre las células de la hipófisis, causando la liberación de las gonadotropinas FSH y LH.

El centro tónico es responsable de la secreción basal de GnRH. Las neuronas en este centro liberan pequeños pulsos de GnRH durante períodos prolongados de tiempo, constituyendo la liberación tónica de GnRH que se produce durante todo el ciclo estral. El centro pulsátil es responsable de la liberación preovulatoria de GnRH, que en contraste con la liberación tónica, se produce una sola vez al final del ciclo. Antes de la instauración de la pubertad, el centro tónico tiene una alta sensibilidad a la retroalimentación negativa inducida por el estrógeno, de modo que la secreción de GnRH permanece inhibida. Durante el período inmediato anterior a la pubertad, la teoría predominante es que se produce una disminución en la sensibilidad del centro tónico a la retroalimentación negativa inducida por el estradiol, permitiendo un incremento en la secreción de GnRH (hipótesis gonadostática). El aumento en la secreción de GnRH incrementa a su vez la secreción de LH (Pelletier et al., 1981; Prunier et al., 1993) en condiciones de elevado estradiol sanguíneo (Lutz et al., 1984; Camous et al., 1985). Cuando las concentraciones de estrógeno en sangre alcanzan un determinado nivel, el centro pulsátil se estimula positivamente liberando una mayor cantidad de la GnRH. Esto estimula el pico de LH que causa la primera ovulación y el inicio de la pubertad. Aunque hay numerosas pruebas que apoyan esta teoría (Berardinelli et al., 1984; Lutz et al., 1984), otros

no han podido demostrar una reducción en la retroalimentación negativa al estradiol antes de la primera ovulación (Elsaesser et al., 1991).

2.2.3 Dinámica ovárica antes de la pubertad:

Los folículos antrales se observan histológicamente por primera vez aproximadamente a los 65 días de edad, y emergen de la superficie del ovario a los 80 días (Black and Erickson, 1968). La formación y el aumento en el número de folículos antrales alrededor de los 100 días es probablemente debido a un aumento transitorio en la secreción de LH (Evans y O'Doherty, 2001) y FSH (Camous et al., 1985) durante este intervalo de tiempo. Aunque los folículos antrales formados durante el período prepuberal poseen la capacidad potencial de ovular, todos ellos sufren atresia después del día 120, coincidiendo con una disminución del 60% en la media de la concentración plasmática de FSH y LH (Guthrie y Garret 2000). Sólo aquellos folículos desarrollados al final de la fase prepuberal son capaces de completar su desarrollo final.

La existencia de un intenso recambio folicular entre los dias 140 y 180 del periodo prepuberal ha sido demostrada en cerdas nulíparas mediante laparoscopia repetida cada 20 dias (Grasso et al., 1988). En un estudio, Bolamba et al., (1994) mostro cambios en el aspecto morfológico de los ovarios, que en un plazo de pocos días cambiaban de un tipo definido como "panel de abeja" (con un gran número de folículos de pequeño diámetro), a un tipo llamado “racimo de uva " (con varios folículos grandes), demostrando que en cerdas prepúberes existen olas de crecimiento folicular similares a las novillas (Evans et al., 1994; Melvin et al., 1999). Sin embargo, en contraste con las novillas, los cambios foliculares en las cerdas prepúberes parecen estar regulados por factores intraováricos locales, en vez de por las

gonadotropinas hipofisarias y el estradiol (Bolamba et al., 1994), ya que en este experimento se observó asincronía en la morfología ovárica entre el ovario izquierdo y el derecho del mismo animal.

2.3 Perfiles hormonales y desarrollo ovárico después de la pubertad

2.3.1 Secreción de GnRH:

En la cerda cíclica los centros tónico y pulsátil están regulados por los niveles de progesterona y estradiol en sangre. La progesterona ejerce una retroalimentación negativa en ambos centros, aunque la mayor parte de su efecto se lleva a cabo sobre el centro tónico. En contraste, el centro pulsátil responde a un aumento en los niveles de estradiol con una retroalimentación positiva. Durante la fase luteal (con altos niveles de progesterona en sangre) la secreción de GnRH por parte del centro tónico ocurre en episodios cada 4-8 h (Senger, 2003). Esta secreción basal, aunque permite un cierto crecimiento folicular, no permite un desarrollo folicular suficiente para la producción de niveles preovulatorios de estradiol. En consecuencia, las cerdas no ovulan bajo la influencia de la progesterona, tanto durante la fase luteal del ciclo estral como durante la gestación.

Durante la fase folicular temprana, la retroalimentación negativa ejercida por la progesterona en el hipotálamo se elimina, y la GnRH es liberada por el centro tónico con una mayor frecuencia que durante la fase luteal (1.5-2 h). Esto estimula el aumento de la liberación de FSH y LH, que a su vez aumenta la secreción de estradiol por parte de los folículos. Durante la fase folicular tardía, y una vez que el estradiol alcanza un cierto nivel en sangre, el centro pulsátil es estimulado positivamente, liberando grandes cantidades de GnRH

que causan la liberación de la ola preovulatoria de LH, tal y como se ha descrito anteriormente para la establecimiento de la pubertad.

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2.3.2 Secreción de LH:

En el cerdo, la vaca y la oveja, la secreción pulsátil de LH cambia de un modo de baja frecuencia y gran amplitud durante la fase lútea, a un modo de alta frecuencia y baja amplitud durante la fase folicular (Clarke, 1989). Estudios en ovejas acerca de la secreción de GnRH muestran que el aumento en la frecuencia pulsátil de LH en plasma podría reflejar la eliminación de la retroalimentación negativa de la progesterona a nivel hipotalámico (Clarke et al., 1987). Por el contrario, Guthrie and Bolt (1990) y otros no han podido demostrar un aumento en la concentración, frecuencias o amplitud de la LH en las cerdas que experimentan luteolisis de forma natural.

A medida que la fase folicular progresa, la amplitud de los pulsos de LH varía entre las diferentes especies (Clarke, 1989). En los cerdos, el crecimiento de los folículos ováricos provoca un aumento progresivo de las concentraciones plasmáticas de estradiol que provoca una inhibición a corto plazo de la secreción de LH durante la fase folicular tardía del ciclo estral (Prunier et al., 1987; Clarke, 1989). De hecho, experimentos en modelos experimentales confirman que los estrógenos pueden ejercer una retroalimentación negativa a corto plazo a nivel pituitario (Britt et al., 1991). En este experimento, los niveles plasmáticos de LH en cerdos ovarectomizados que recibieron una sola inyección de estrógenos mostraron una primera respuesta de retroalimentación negativa y, a continuación una respuesta de retroalimentación positiva. Aparentemente, estradiol puede inhibir o reducir en gran medida la liberación de GnRH por un período de aproximadamente 54 hasta 60 h , y a continuación, provocar una liberación masiva de GnRH, induciendo una ola preovulatoria de LH

proporcional a la cantidad de la GnRH secretada. La importancia fisiológica de esta inhibición temporal podría ser el darle tiempo a las células hipofisarias para preparar la maquinaria celular para el subsiguiente evento de retroalimentación positiva.

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2.3.3 Secreción de FSH y dinámica folicular:

En porcino, cambios en las concentración de esteroides en el líquido folicular indican dos olas de crecimiento folicular y aumento de la esteroidogénesis (Guthrie y Cooper, 1996), aunque ambas olas no se desarrollan en la misma medida que en el ganado vacuno probablemente debido a la supresión de la secreción de LH por parte de la progesterona ovárica. En el cerdo, el receptor de LH aparece en una etapa relativamente inmadura del desarrollo folicular (~4 mm de diámetro; Liu et al., 1998 ,2000), mientras que los receptores de LH en la vaca no aparecen hasta que los folículos alcanzan 9 a 10 mm de diámetro (Xu et al., 1995). Esto explicaría por qué en la vaca existen ondas foliculares con la aparición de folículos dominantes durante la fase lútea, mientras que los folículos del cerdo no crecen más allá de 4 mm (Driancourt et al., 2001). La primera ola de crecimiento folicular consiste en la reposición de la población de folículos entre los días 2 y 8 del ciclo estral, seguido por un aumento en la incidencia de atresia entre los folículos de pequeño y mediano diámetro entre los días 5 y 7. La segunda ola de crecimiento folicular consiste en la selección y el crecimiento de los folículos preovulatorios, acompañado de la atresia del resto de los folículos durante la fase folicular del ciclo (días 15-21). El patrón de crecimiento folicular durante las etapas media y final de la fase luteal en cerdos (días 7-15) se caracteriza por el continuo crecimiento y atresia de los folículos ováricos (Guthrie and Cooper, 1996). En vacas, yeguas y ovejas, la aparición de nuevas olas de crecimiento folicular es precedida por un aumento transitorio en la secreción de FSH en plasma. En los cerdos, cambios en el número y distribución de tamaño de los folículos durante el ciclo estral están también

parcialmente asociados con cambios en las concentraciones de FSH plasmática (Guthrie et al., 1995). La atresia entre la población de folículos de pequeño y mediano diámetro coincide con una disminución en los niveles de FSH a partir de los días 1 o 2 tras la disminución de los niveles plasmáticos de progesterona (Guthrie and Bolt, 1990; Knox et al., 2003). La secreción de FSH queda suprimida hasta el pico preovulatorio de LH, y después vuelve a aumentar transitoriamente entre los días 2 y 3 posteriores de la ovulación (liberación periovulatorio de FSH), tiempo durante el cual los ovarios reponen las poblaciones de folículos pequeños (1-2 mm de diámetro) y medianos (3-6 mm, 35 a 40 por animal; Guthrie, 2005). El incremento en los niveles de atresia entre las poblaciones foliculares pequeñas y medianas entre los días 6 y 7 después de la ovulación coincide con una disminución de la concentración de FSH, y podría ser consecuencia de la retroalimentación negativa por parte de la inhibina sobre la secreción de FSH (Guthrie et al., 1995).

2.3.4 Secreción de estrógeno y progesterona:

El estradiol es secretado por los folículos durante todo el ciclo estral, pero la concentración permanece baja durante la fase luteal debido al efecto inhibidor de la progesterona a nivel hipotalámico. Una vez que se produce la luteolisis y los folículos preovulatorios son seleccionados, los niveles circulantes de estradiol aumentan progresivamente hasta desencadenar la liberación preovulatoria de GnRH. Por el contrario, la concentración de progesterona baja inmediatamente después de la ovulación y aumenta de nuevo entre los días 3 o 4 del ciclo estral. La progesterona alcanza la máxima concentración en sangre entre los días 7 y 12 de la fase luteal, y a continuación, coincidiendo con la regresión del cuerpo lúteo entre los días 14 y15, disminuye rápidamente y permanece baja durante el resto de la fase folicular.

2.3.5 Reclutamiento y selección folicular

Durante la fase de reclutamiento, una cohorte de folículos comienza su fase de crecimiento final, mientras que durante la selección se eligen los folículos preovulatorios y todos los demás sufren atresia. La importancia de las hormonas LH y FSH en el desarrollo folicular cambia a través de estas dos etapas. En la fase de reclutamiento, la FSH desempeña un papel más importante que la LH en el crecimiento de los folículos antrales. Una vez que los folículos entran en la fase de selección, la inhibina y el estradiol producidos por los propios folículos en crecimiento inhiben la secreción de FSH a nivel de la hipófisis, mientras que aumenta la secreción de LH. Por último, los folículos preovulatorios producen más estrógeno, que finalmente estimula la ola preovulatoria de LH.

El reclutamiento y selección folicular son procesos relativamente cortos en comparación con las etapas de desarrollo folicular anteriores. El intervalo de tiempo desde que un folículo primordial inicia su crecimiento hasta que se produce la formación del antro es de 83 días, mientras que desde la formación de antro hasta que el folículo alcanza la fase ovulatoria pasan sólo 20 días (Morbeck et al., 1992). Basándose en esta estimación de la tasa de crecimiento, Morbeck et al. (1992) planteó la hipótesis de que los folículos que comienzan la formación del antro al inicio del ciclo estral pueden llegan a alcanzar un diámetro de 3 mm en el días 14 a 16, constituyendo la población de la que se reclutan los folículos ovulatorios.

Reclutamiento folicular

El término de reclutamiento folicular se ha utilizado para describir dos etapas durante el desarrollo de los folículos. Los folículos primordiales latentes en los ovarios son reclutados

de una manera continua (reclutamiento inicial), mientras que los incrementos en la FSH circulante en cada ciclo estral causan el reclutamiento de una cohorte de folículos antrales (reclutamiento cíclico; McGee and Hsueh, 2000). Durante el reclutamiento inicial, factores intraováricos estimulan algunos folículos primordiales para iniciar el crecimiento, mientras que el resto de los folículos no reclutados permanecen en estado latente. Tras este periodo de reclutamiento, los folículos crecen pero los oocitos permanecen detenidos en la profase de la meiosis. En la fase de reclutamiento cíclico, que comienza después del inicio de la pubertad como consecuencia de los aumentos cíclicos de FSH, se forman cohortes de folículos antrales que comienzan su crecimiento final dependiente de las hormonas gonadotrópicas. A diferencia del caso anterior, durante el reclutamiento cíclico sólo un número limitado de folículos sobrevive, mientras que los demás sufren atresia. Los oocitos en los folículos reclutados han completado su crecimiento, han adquirido una zona pellúcida, y están preparados para reanudar la meiosis (McGee and Hsueh, 2000). El número de folículos reclutados es altamente variable entre las diferentes especies, com más de 50 folículos en los cerdos, de 5 a 10 en el ganado vacuno, y de 1 a 4 en el caballo (Driancourt, 2001). En la cerda, el reclutamiento folicular se produce entre los días 14 y 16 del ciclo estral (Clark et al., 1982; Foxcroft and Hunter, 1985), o poco después del destete. En el día 16, aproximadamente 40 a 50 folículos de 3 a 6 mm de diámetro están presentes en ambos ovarios, y representan la población total de folículos reclutados (Grant et al., 1989).

En el ganado bovino, el inicio de las olas de crecimiento folicular está precedido por un aumento transitorio de la FSH que estimula el desarrollo de los folículos antrales. Sin embargo, en la cerda no se ha detectado un claro incremento en las concentraciones de FSH coincidentes con el período de reclutamiento folicular (Flores et al., 1989; Guthrie and Bolt, 1990; Cárdenas and Pope, 2002), aunque las concentraciones plasmáticas de FSH estan

consistentemente más elevadas que durante el periodo preovulatorio del ciclo. Guthrie and Bolt (1990) sugieren la posibilidad de que no solo los cambios en la concentración individual de FSH, sino la alteración del ratio LH:FSH, podría estar detrás de la regulación del proceso de reclutamiento folicular.

Selección:

Aproximadamente el 30 o 40% de los folículos reclutados se seleccionan para completar la maduración final y la ovulación, mientras que el resto de los folículos y de los oocitos que contienen sufren atresia y desaparecen de los ovarios. Por tanto, el número final de folículos ovulatorios está determinado por la cantidad de folículos reclutados y por la capacidad de estos folículos para seguir creciendo y evitar la atresia durante el proceso de selección (Cárdenas and Pope, 2002). La intensidad del proceso de selección (medido por la proporción de folículos supervivientes en la cohorte ovulatoria) es muy variable entre las especies, siendo muy baja en caballos, que tienen un pequeño tamaño de cohorte, mientras que es muy alta para el ganado bovino (un folículo seleccionado entre 5) y porcino (12 folículos seleccionados de una cohorte de 50; Driancourt, 2001).

El mecanismo de selección folicular difiere entre el ganado bovino y porcino, debido a los diferentes receptores foliculares presentes en el momento de la selección (Liu et al., 1998; 2000; Xu et al., 1995) **(Figura 2.4)**. Lucy (2007) propuso dos modelos diferentes para describir el proceso de selección folicular. Ambos modelos sugieren que un folículo tiene una ventaja en su desarrollo sobre los demás en el momento de la selección folicular. Esta ventaja podría ser conferida por aspectos individuales del folículo en el comienzo de la onda

folicular, incluyendo su lecho vascular, o el número o el estado de las células de la granulosa y de la teca.

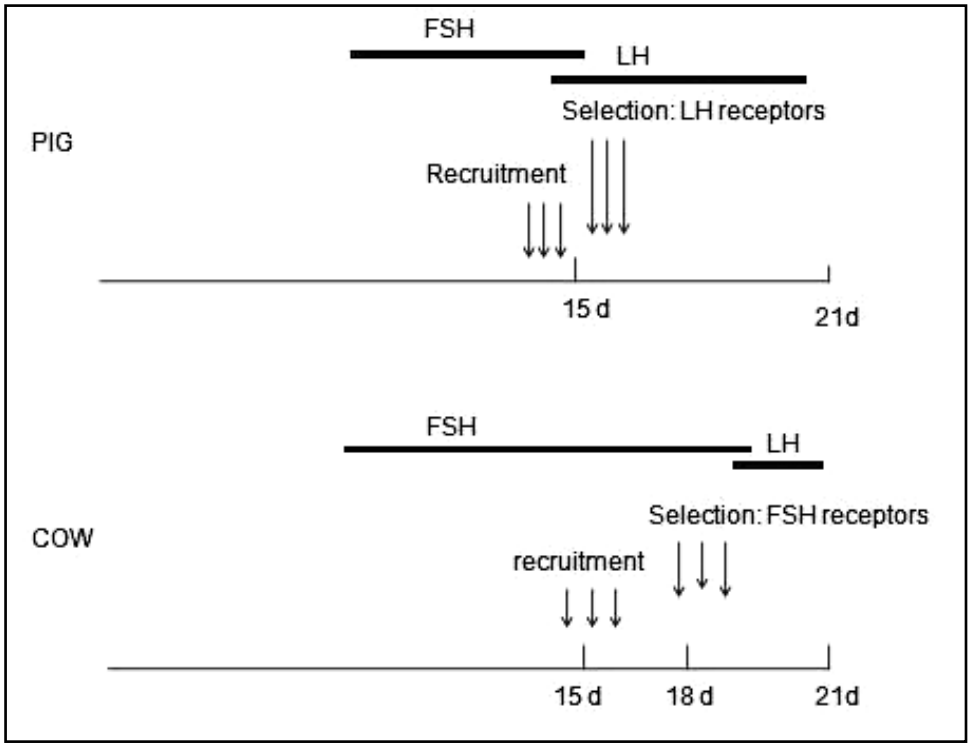

Figura 2.4 Mecanismos de selección folicular en ganado bovino y porcino (basado en información tomada de Lucy, 2007 y Driancourt, 2001).

De acuerdo con el modelo de Missouri (Xu et al., 1995), los primeros folículos en desarrollar receptores para LH teóricamente podrían causar atresia en los demás folículos a través de la inhibición selectiva de la FSH. Este modelo podría explicar el mecanismo de selección en la cerda, debido a que el desarrollo del receptor de LH en células de la granulosa se produce en una fase temprana del crecimiento folicular preovulatorio (Liu et al., 1998; 2000), permitiendo a una cohorte de folículos responder a la LH cuando las concentraciones de FSH en sangre disminuyen, escapando de este modo de la atresia. Por el contrario, en las vacas la adquisición de receptores de LH por parte de las células de la granulosa no es un

componente clave de selección folicular, ya que ocurre casi al final del crecimiento preovulatorio (Fortune et al., 2001).

De acuerdo con el modelo de Cornell (Fortune et al., 2004), el aumento de la concentración de FSH durante la proceso de reclutamiento folicualr incrementa la síntesis de la enzima que degrada la proteína de unión al factor de crecimiento similar a la insulina tipo I (IGF BP) presente en el líquido folicular, y por tanto conduce a un aumento de IGF-I libre. El aumento en la concentración de IGF-I incrementa la actividad esteroidegenica intrafolicular de FSH, asi como la actividad de LH, aumentando la tasa de crecimiento del folículo dominante y la concentración de estradiol en sangre. Además, el aumento en la concentración de folistatina haría que esta se una a la activina folicular, cambiando el equilibrio inhibina/activina hacia la inhibina. La inhibina producida dentro de la capa de células de la granulosa también potencia la acción de la LH a nivel de las células de la teca. Finalmente, el aumento de los niveles de estradiol disminuye la concentración de FSH causando la atresia de todos los folículos restantes. Este modelo podría explicar la selección folicular en vacas, puesto que a diferencia del cerdo, el receptor para la FSH continúa siendo expresado en la capa de células granulosa durante todo el periodo folicular del ciclo estral (Xu et al., 1995). Respecto al desarrollo de receptores de LH en las células de la granulosa en las vacas, se ha planteado la hipótesis de que este mecanismo permitiría a las células de la granulosa aumentar la actividad de la aromatasa en respuesta a la FSH y LH, y de esta forma aumentar, o al menos mantener, su capacidad para producir más estradiol que los folículos subordinados. Otra posibilidad es que los receptores de LH se desarrollen en células de la granulosa de los folículos dominantes con el fin de prepararlos para su posterior diferenciación en respuesta al pico de LH. (Fortune et al., 2004).

2.4 Estrategias para la estimulación de la pubertad en cerdas prepúberes

El número de cerdos destetados semanalmente depende principalmente de la inseminación de un número suficiente de hembras cada semana (Dial et al., 1996). Con el fin de tener suficientes hembras disponibles, es necesario disponer de un suministro garantizado de cerdas de reposición. Este objetivo se consigue mediante la estimulación temprana de la pubertad y el mantenimiento de una actividad cíclica normal posterior a la primera ovulación. Los únicos métodos eficaces para la estimulación prematura de la pubertad son la exposición al verraco y la inyección de las hormonas gonadotrópicas.

2.4.1 Exposición al verraco:

La exposición a un verraco es la práctica más común para la estimulación temprana de la pubertad. Una estimulación adecuada del celo requiere el contacto físico directo entre el macho y las cerdas prepúberes, mientras que para la detección del celo solo es necesario un contacto a través de la valla de separación. Para garantizar la eficacia de la exposición al verraco, es importante seguir unas reglas mínimas (Kirkwood and Thacker 1992; Hughes, 1997):

1. Las cerdas jóvenes deberán tener por lo menos 160 días de edad, aunque estudios recientes sugieren que es preferible que tengan 180 días.
2. Los verracos deberán tener al menos 10 meses de edad. Una parte importante del estímulo del macho consiste en la producción de feromonas por la glándula salival submaxilar, y esta glándula no se desarrolla completamente hasta que el animal alcanza los 9 o 10 meses.
3. Las cerdas prepúberes deben estar en contacto físico con el jabalí durante al menos 15 minutos por día.

4. La exposición al macho debe realizarse al menos dos veces al día en un espacio suficientemente grande (>1.5 m^2 por cerda joven), ya que el hacinamiento de los animales puede retrasar la pubertad y hace más difícil la detección de celos.
5. Las cerdas prepúberes deben alojarse al menos a 1 m de distancia de los verracos para evitar que se acostumbren a los estímulos del macho, causando problemas en la detección del celo.
6. Si las hembras no responden como se esperaba, es aconsejable la utilización de un macho diferente.
7. Si las cerdas no se inseminan en su primer celo, la exposición al verraco debe seguir al menos durante 5 minutos cada día con el fin de promover los ciclos estrales regulares. En ausencia de una exposición continuada, muchas hembras desarrollan intervalos de celo irregulares.

2.4.2 Utilización de hormonas exógenas:

Si la exposición al verraco no resulta eficaz, por ejemplo debido a un efecto estacional, la inducción del celo mediante hormonas exógenas se vuelve necesaria. Combinaciones comerciales de hormonas gonadotrópicas contienen la gonadotropina coriónica equina (eCG), que puede ser usada sola o en combinación con la gonadotropina coriónica humana (hCG). Una combinación hormonal muy común en porcino es 400 UI de eCG + 200 UI de hCG (PG600®; Intervet). La inyección de PG600 es eficaz para la inducción del estro en cerdas destetadas, pero cuando se administra a las cerdas prepúberes para la estimulación de la pubertad, hasta el 30% de los animales puede no exhibir comportamiento estral, y aproximadamente el 30 % de aquellos que exhiben signos de estro pueden no ovular regularmente (Tabla 2.1).

Tabla 2.1: Porcentaje de celos en cerdas prepúberes en respuesta a la inyección con PG600[1]

Estrous (%)	Ovulation (%)	Cycling (%)	Source
88	100	87	Paterson (1982)
--	97	60	Paterson (1982)
70	99	--	Tilton *et al*. (1995)
78	--	67	Kirkwood (1999)

[1]PG600: combinación de 400 IU eCG + 200 IU hCG (Intervet International)

La etiología de la falta de respuesta a la inyección con PG600 es desconocida, aunque es posible que se deba a la hormona hCG de la preparación, que induce una ovulación inmediata en los animales o la luteinización de los folículos ováricos, causando una producción prematura de progesterona que suprimiría el comportamiento estral de los animales. Por lo tanto, es necesario el desarrollo de nuevos protocolos hormonales que mejoren las tasas de celo y ovulación en cerdas prepúberes. Sin embargo, con el fin de lograr este objetivo, primero es necesario determinar por qué el 30% de las cerdas no responden a la inyección de PG600.

CAPÍTULO 3: Effect of eCG or eCG plus hCG on Estrus Expression and Ovulation in Prepubertal Gilts

Published in Reproduction in Domestic Animals, 2009; 44(3):411-413

Reprod Dom Anim **44**, 411–413 (2009); doi: 10.1111/j.1439-0531.2008.01089.x
ISSN 0936-6768

Effect of eCG or eCG Plus hCG on Oestrus Expression and Ovulation in Prepubertal Gilts

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Contents

To meet weekly breeding targets, it is occasionally necessary to inject exogenous gonadotrophins to induce oestrus in prepubertal gilts. However, the gilt oestrus response to equine chorionic gonadotrophin (eCG) either alone or in combination with human chorionic gonadotrophin (hCG) can be unpredictable. The objective of the present study was to examine possible reasons for this unpredictability. Prepubertal gilts (90 kg and 153 days of age, n = 109) received an injection of either 600 IU eCG or a combination of 400 IU eCG and 200 IU hCG (PG600), or were non-injected controls, and were then exposed to a mature boar for 15 min daily for 7 days for oestrus detection. At the time of injection, real-time ultrasound revealed that the gilt ovaries had primarily 1–2 mm follicles. Blood samples were obtained at time of hormone injection (day 0) and at days 3, 7 and 10 for assay of serum progesterone concentrations. The oestrus responses by 7 days were15.5%, 73.3% and 0%, for eCG, PG600, and control gilts, respectively ($p < 0.001$). The oestrus response improved ($p < 0.05$) with increasing body weight. Based on circulating progesterone levels, all oestrous gilts ovulated except for four of the PG600 gilts. Failure to express oestrus in PG600 gilts was not associated with a premature rise in progesterone.

Introduction

The largest single component of herd non-productive days is often the gilt entry-to-service interval. To minimize this interval, gilts should reach puberty as soon as possible either after arrival on the farm or entry into the breeding herd. The ability to meet the weekly breeding target requires a predictable supply of service-ready gilts (i.e. gilts in oestrus when required), which is most easily achieved by having gilts show an early puberty and then maintain predictable cyclic activity. When gilt availability is limiting, a common method used to stimulate the onset of oestrus is the injection of gonadotrophins, such as the combination of 400 IU eCG and 200 IU hCG (PG600®). While this product is effective for the induction of a fertile oestrus in prepuberal gilts, in practice up to 30% of treated gilts do not show a behavioural oestrus response within 7 days (Kirkwood 1999). However, based on blood progesterone determinations at 10 days after treatment, most non-responding gilts do appear to have ovulated (Tilton et al. 1995).

Taking the above findings into consideration, we hypothesized that the 30% non-oestrous response is due to the hCG component of PG600, the hCG being a LH analogue causing some gilts to either ovulate or suffer luteinization of medium or large follicles. In either event, the premature production of progesterone would block behavioural oestrus and the gilts will be deemed to have failed to respond to treatment. We further suggest that if our hypothesis were true, then injection of eCG alone (i.e. no hCG component) would result in an improved oestrus response because used alone, this molecule would provide a much lower LH activity (Combarnous et al. 1984; Guthrie et al. 1990).

Materials and Methods

These studies were performed during October and November 2006 at Michigan State University Swine Facility with the approval of the institutional Animal Care committee.

To examine responses to different gonadotrophin preparations, 109 prepubertal Yorkshire x Landrace gilts (90 kg, 153 days) were employed. Gilts were housed at 8–12 animals per pen and fed *ad libitum* a corn-soybean meal finisher diet formulated to provide 13.7 MJ ME/kg, 9.8% crude protein, 0.6% lysine. At selection, gilts were assigned by weight and age to receive an intramuscular (IM) injection of 600 IU eCG (Pregnecol®; Bioniche Animal Health, Belleville, ON, Canada, n = 45) or 400 IU eCG plus 200 IU hCG (PG600®; Intervet, Millsboro, Delaware, n = 45). A third group of gilts served as uninjected controls (n = 19). All treatments were represented in each pen. The dose of eCG (600 IU) was chosen on the basis of it being a label dose for sows at weaning and that it is the 'total' gonadotrophin content of PG600. From 2 days until 7 days after the time of hormone injection, all gilts were subject to direct exposure to a mature boar for 15 min daily to facilitate detection of oestrus**.** Oestrus was defined as the expression of a rigid standing reflex in the presence of the boar. Fewer control gilts were employed because we anticipated few, if any, would exhibit oestrus during the 7 days study period.

To further characterize the oestrus and ovulation responses to hormone treatment, blood samples were obtained from all gilts via jugular venipuncture at the time of hormone injection (0 day) and at 3, 7 and 10 days. Serum samples were assayed for progesterone (P4) concentrations in a single assay using a commercial kit (Diagnostic Product Corp., Los Angeles, California). Assay sensitivity and intra-assay coefficient of variation were 0.1 ng/ml and 2.7%, respectively. All gilts had non-detectable P4 concentrations on 0 day. An elevation in P4 to >1 ng/ml on 3 days was considered indicative of premature ovulation (or luteinization), while low levels on 3 days followed by elevations on 7 days and/or 10 days was taken to indicate a normal ovulatory response.

Immediately prior to hormone injection, the first 25 gilts assigned to each hormone treatment were subject to transrectal B-mode ultrasonography of their ovaries using an Aloka SSD 500 (Aloka Inc., Wallingford, CT, USA) with a 7.5-MHz linear array transducer as described by Knox and Althouse (1999). The diameters of the largest three follicles were recorded.

Data were analysed using the Number Cruncher Statistical System (NJ Hintze, Kayeville, UT, USA). Treatment differences on the oestrus response rates were examined by Chi square and differences in age, weight and follicle diameter tested by GLM analysis of variance.

Results

There were no differences between treatments for gilt age or weight at the start of the study (Table 1). Similarly, there was no difference in ovarian follicular diameter prior to hormone injection (Table 1). Most gilts had a maximum follicular size of 2 mm although 3 mm follicles were measured in seven of the eCG-treated and five of the PG600-treated gilts, and two of the PG600-treated gilts had 4 mm follicles. The PG600-treated gilts with the largest follicle ovulated normally.

As anticipated, no control gilts exhibited oestrus during the study period, and none had elevated serum P4 concentrations. Contrary to expectation, more ($p < 0.001$) PG600-treated gilts than eCG-treated gilts exhibited oestrus by 7 days after injection (Table 1). All eCG-treated gilts exhibiting oestrus had serum P4 concentrations indicative of normal ovulation. Elevated P4 concentrations were not detected in any anoestrus eCG-treated gilt. Of the 33 (73%) PG600-treated gilts exhibiting oestrus, four failed to ovulate. Of the 12 anoestrus PG600-treated gilts, none had elevated P4 on day 3 after injection and only one had serum P4 concentrations indicating a normal ovulation.

There was an effect of body weight at treatment on the incidence of oestrus, with more of the gilts weighing >90 kg exhibiting oestrus than their lighter counterparts ($p < 0.05$). However, because too few eCG-treated gilts exhibited oestrus, the effect was only significant for PG600-treated gilts (Table 2). The two weight classes did not differ in age (154.9 ± 5.0 vs 157.5 ± 3.8 days for the lighter and heavier gilts, respectively, $p = 0.8$).

Table 1. Influence of eCG or eCG plus hCG on incidence of oestrus by 7 days and ovulation as indicated by elevated progesterone (+P4) or non-ovulation (–P4)

	eCG + hCG	eCG	Control
No. of gilts	45	45	19
Age (days)[a]	154.1 ± 2.6	154.2 ± 2.7	150.6 ± 3.8
Weight (kg)[a]	90.1 ± 1.3	91.4 ± 1.3	88.5 ±2.0
Follicle diameter (mm)[a]	2.4 ± 0.1	2.3 ± 0.1	–
Gilts oestrus by 7 days (%)[b]	73.3	15.6	0
Gilts oestrus + P4 (%)	64.4	15.6	–
Gilts oestrus – P4 (%)	8.9	0	–
Gilts anoestrus + P4 (%)	2.2	0	–
Gilts anoestrus – P4 (%)	24.4	84.4	–

[a]Mean ± SE.
[b]Effect of hormone preparation, $p < 0.001$ by chi-square test.

Table 2. Influence of gilt weight on the oestrus response to eCG or eCG plus hCG

	eCG + hCG	eCG	Control
Number of gilts	45	45	19
75–90 kg	13⁄22 (59.1%)[a]	2⁄23 (8.6%)	0
91–110 kg	20⁄23 (86.9%)[b]	5⁄22 (22.7%)	0

[ab]Effect of weight, $p < 0.05$ by ANOVA.

Discussion

The results of this study confirmed an approximately 70% oestrus response to PG600 but did not support the hypothesis that injection of hCG concurrent with eCG results in follicular luteinization or ovulation and premature elevation of circulating progesterone. Indeed, the poor oestrus response to eCG compared to the eCG⁄hCG combination suggests an important role for the hCG in follicular development and subsequent oestrus expression. However, a follicular ovulatory or luteinizing effect may depend on the follicular status of the ovaries at the time of stimulation (i.e. presence or not of medium to large follicles). In the present study, few gilts had follicles >2 mm in diameter.

Of the PG600-treated gilts exhibiting oestrus, four of them did not have elevated circulating progesterone levels. It is possible that these gilts underwent sufficient follicular development to cause circulating oestrogen levels adequate to induce behavioural oestrus, but then failed to ovulate. A failure to ovulate may be due to the PG600 activity being inadequate to complete follicular development to the point of inducing the phasic LH release. However, in the absence of measurement of circulating LH concentrations and ongoing follicular ultrasound examinations, these suggestions remain speculative.

It is probable that the response to eCG treatment, with or without concurrent hCG treatment, will depend on the gilt's level of physiological maturity, which is supported by the positive association between gilt weight and their oestrus response in the present study, although gilt age was not a significant factor. Most of the gilts in the present study had only small (1–2 mm) follicles on their ovaries, which should have been responsive to FSH-like stimulation (Guthrie et al. 1990), although the possibility that they were too immature to respond to gonadotrophic stimulation cannot be discounted. However, it was shown previously that the presence of many or only a few large (>6 mm) follicles on the ovary did not affect the oestrus or ovulatory response of prepubertal gilts to eCG treatment. This suggests that eCG can stimulate development of small follicles to the point of ovulation (Bolamba et al. 1992).

It is possible that a period of FSH stimulation would advance follicular development (Guthrie et al. 1988; Bolamba et al. 1996) such that they would then be more responsive to eCG stimulation. Alternatively, it is possible that some minimum level of LH-like stimulation is necessary to complete follicular development (Driancourt et al. 1995). Previous studies examining oestrus and⁄or ovulatory responses to eCG have yielded

variable results. Response rates of 70–100% have been noted when 725–1000 IU eCG was injected (Guthrie 1977; Dial et al. 1984; Britt et al. 1986; Esbenshade 1987; Flowers et al. 1989; Bolamba 1992) although oestrus rates of only 25% to 52% were noted following injection of 363–600 IU (Britt et al. 1985; do Lago et al. 2005; Gama et al. 2005). The difference in responses may be due to the degree of physiological development and genetics of the gilts and/or the source of the eCG, although it is likely that the greater LH-like activity associated with higher doses improved the likelihood of ongoing follicular development.

We conclude from the present data that the gilt oestrus responses to eCG will be improved with concurrent use of hCG and that, depending on their degree of physiological development, a failure of gilts to exhibit oestrus in response to a combination of eCG and hCG need not involve premature ovulation or luteinization of follicles and an associated premature elevation in circulating progesterone.

Acknowledgements

Hormones were generously provided by Bioniche Animal Health, Bellville, Ontario and Intervet, Milsboro, Delaware. Financial support for this study was provided by Michigan State University.

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Submitted: 17 Nov 2007

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CAPÍTULO 4: Effect of Prior FSH Treatment on the Estrus and Ovulation Responses to eCG in Prepubertal Gilts

Published in Animal Reproduction Science, 2009; 110(1-2): 123-127

ELSEVIER

Animal Reproduction Science 110 (2009) 123–127

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Effect of prior FSH treatment on the estrus and ovulation responses to eCG in prepubertal gilts

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Received 29 October 2007; accepted 8 January 2008
Available online 15 January 2008

Abstract

The objective of this study was to determine the effect of pre-treatment of prepubertal gilts with FSH on the estrus and ovulatory responses to eCG injection at two ages. A total of 149 prepubertal Hypor gilts were selected at 150 days ($n = 76$) or 180 days ($n = 73$) of age and assigned to injection of 400 IU eCG plus 200 IU hCG (PG600), 600 IU eCG alone (Folligon), pre-treatment with 72 mg FSH (Folltropin) administered as 6 × 12 mg injections at 12 h intervals with 600 IU Folligon 12 h after last FSH injection, or non-injected controls. To facilitate detection of estrus, gilts were exposed to a mature boar for 15 min daily for 7 days. To determine ovulatory responses, blood samples were obtained on the day of injection and 10 days later and assayed for progesterone content. Following treatment at 150 days, one control gilt (5.3%) was deemed estrus but ovulation did not occur. Compared to treatment with Folligon alone, PG600 injection tended ($P = 0.1$) to increase the estrus response (52.6% compared with. 26.3%) and increased ($P < 0.01$) the ovulatory response (89.5% compared with. 47.4%). The estrous response in gilts pretreated with Folltropin was intermediate (42.1%) but the ovulatory response (47.4%) was the same as for Folligon alone. Following treatment at 180 days, two control gilts (10.5%) were deemed estrus and ovulation did occur in these gilts. There was no difference between hormone-treated groups for estrus or ovulatory responses, although the ovulatory response of PG600-treated gilts tended ($P = 0.1$) to be greater than for the Folligon-treated group (89.5% compared with 66.7%), with Folltropin-pretreated gilts being intermediate (76.5%). These data demonstrate that the estrus and ovulatory responses of gilts were greater for PG600 than for Folligon and that while

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0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2008.01.001

responses to PG600 were not affected by gilt age, for the combined Folligon groups, estrous response ($P < 0.02$) and ovulatory response ($P < 0.05$) improved with increased gilt age.

Keywords: Gilts; FSH; eCG; Estrus; Ovulation

1. Introduction

To minimize the gilt entry-to-service interval, or where availability of service-ready gilts is limiting, gilts are often induced into estrus by injection of gonadotropic hormones. A common preparation used for induction of estrus is a combination of 400 IU equine chorionic gonadotrophin (eCG) and 200 IU of human chorionic gonadotrophin (hCG; PG600®). Subsequent to injection of PG600, it is not unusual to have only 70% of gilts show a behavioral estrous response within 7 days (Kirkwood, 1999). It was hypothesized that the 30% failure to respond to PG600 was due to its hCG component luteinizing or ovulating medium or large follicles, causing a premature increase in circulating progesterone and blockade of behavioral estrus (Manjarin et al., 2008). However, in this earlier work we did not observe any indication of ovulation or follicular luteinization subsequent to PG600 injection into 150-d old prepubertal gilts. Interestingly, when 600 IU eCG alone was injected, it resulted in only a 15% estrous response in prepubertal gilts (Manjarin et al., 2008). The poor response to this relatively small dose of eCG may have been due to the immaturity of ovarian follicles (90% <3 mm), indicating a need for the concurrent biological activity of hCG to adequately stimulate growth of small follicles. This is supported by the improved estrous response observed with larger doses of eCG (Dial et al., 1984; Britt et al., 1985), which will also have a greater LH-like activity (Dial et al., 1984).

Interestingly, while injection of eCG into prepubertal gilts caused atresia in the small and medium follicle populations, injections of FSH promoted follicular growth (Guthrie et al., 1990; Bolamba et al., 1996; Guthrie, 2005). Also, changes in follicle number and size during prepubertal development were temporally associated with changes in circulating FSH concentrations (Camous et al., 1985; Guthrie and Garrett, 2000). Further, suppression of FSH during the luteal phase of post-pubertal gilts decreased the small and medium follicle populations (Knox and Zimmerman, 1993; Guthrie et al., 1987), which were restored by FSH replacement (Guthrie et al., 1988). Taking the above all together, we hypothesize that administration of FSH to prepubertal gilts will increase the populations of medium sized ovarian follicles, which will enhance the gilts ovarian response to eCG injection. Ovarian follicle diameters were not measured in the present study, thus a positive effect of FSH on rates of estrus and ovulation were taken to indicate support for the hypothesis.

2. Materials and methods

This study was performed on a commercial 1000-sow farrow-to-wean facility near Leon, Spain during July and August 2006. Animals were cared for humanely in accordance with institutional animal care guidelines. A total of 149 Hypor gilts were used to examine responses to different gonadotropin preparations. Gilts were housed at 6–8 animals per pen and fed *ad libitum* a standard wheat/soybean meal finisher diet formulated to supply 14 MJ DE/kg and 0.8% lysine. Gilt weights and backfat depths were not available on this farm so they were assigned by age (150 or 180 days) to receive an intramuscular (IM) injection of 400 IU eCG plus 200 IU hCG (PG600®, Intervet

International, Boxmeer, NL; $n = 19$ and 19, at 150 and 180 days, respectively), 600 IU eCG (Folligon®, Intervet; $n = 19$ and 18, respectively), or pre-treatment with 72 mg FSH (Folltropin®; Bioniche Animal Health, Bellville, Ontario, $n = 19$ and 17, respectively) followed by 600 IU eCG. The FSH was administered as 6×12 mg injections at 12 h interval with the eCG being administered 12 h after final FSH injection. A fourth group of gilts served as uninjected controls ($n = 19$ and 19, respectively). From 2 to 7 days after hormone injection, gilts were subject to direct exposure to a mature boar for 15 min daily to facilitate detection of estrus.

To determine the ovulation responses to hormone treatment, blood samples were obtained from all gilts via jugular venipuncture at the time of hormone injection and 10 days later. Serum was harvested and frozen (−20 °C) until assayed for progesterone concentrations. Serum samples were assayed for progesterone concentrations in a single assay using a commercial ELISA kit (Immulite®, Siemens Medical Solutions Diagnostics, Tarrytown, NY). Assay sensitivity and intra-assay coefficient of variation were 0.2 ng/ml and 8.1%, respectively. All gilts employed in this study had non-detectable progesterone concentrations on the day of injection. An elevation of progesterone concentrations on day 10 to a minimum of 4 ng/ml was deemed to indicate an ovulatory response to treatment. The effects of treatment and gilt age on the proportional estrus and ovulation responses were examined by Chi square. For the determination of age effects, the eCG and FSH followed by eCG groups were combined.

3. Results

A single 150 days control gilt (5.3%) was detected as being estrus but ovulation did not occur in this gilt (Table 1). When compared to the gilts treated with eCG alone, PG600 injection tended ($P = 0.1$) to increase the estrous response (52.6% compared with 26.3%) and increased ($P < 0.01$) the ovulatory response (89.5% compared with. 47.4%). The estrous response in eCG-treated gilts that were pre-treated with FSH was intermediate (42.1%) but their ovulatory response (47.4%) was the same as for eCG alone. Following treatment at 180 days, two control gilts (10.5%) were deemed estrus and ovulation was detected in these gilts. There was no difference between any hormone-treated group for estrous or ovulatory responses, although the ovulatory response of PG600-treated gilts tended ($P = 0.1$) to be greater than for the gilts receiving eCG alone (89.5% compared with 66.7%), with the FSH-pretreated gilts being intermediate (76.5%). There was no effect of age on estrous or ovulatory responses of PG600-treated gilts but the estrous and ovulatory responses to eCG were greater in older gilts ($P < 0.05$ for both).

Table 1
Influence of gilt age (150 compared with 180 days) on the estrus and ovulation responses to eCG plus hCG combination, eCG alone, FSH pre-treatment then eCG, or no treatment

	eCG + hCG	eCG	FSH + eCG	Control
150 days				
No. of gilts	19	19	19	19
Gilts estrus by 7 days	10 (52.6%)	5 (26.3%)	8 (42.1%)	1 (5.3%)
Gilts ovulating	17 (89.5%)	9 (47.4%)	9 (47.4%)	0
180 days				
No. of gilts	19	18	17	19
Gilts estrus by 7 days[a]	13 (68.4%)	12 (66.7%)	10 (58.8%)	2 (10.5%)
Gilts with ovulations	17 (89.5%)	12 (66.7%)	13 (76.5%)	2 (10.5%)

[a] One estrous-eCG + hCG-treated gilt failed to have an ovulation.

4. Discussion

The primary objective of the present study was to test the hypothesis that gilts pre-treated with FSH would have greater estrous and ovulation responses to eCG injection, presumably due to enhanced follicular development. The hypothesis is not supported because no difference in proportions of gilts having ovulations between the two eCG-treated groups was observed. Although ovarian follicular dynamics was not assessed in the present study, the FSH treatment protocol was similar to others where an effect on follicle growth was evident (Guthrie et al., 1990; Bolamba et al., 1996). This suggests that even if FSH was able to stimulate follicular growth to the medium follicle size, these follicles were not more responsive to eCG at either 150 or 180 days of age. It is probable that the ovarian response to eCG stimulation will depend on the gilt's stage of physiological maturity, which will include the entire hypothalamo-pituitary-ovarian axis, not just at the ovary. This is supported by a positive association between gilt weight and their estrous response noted in our earlier study (Manjarin et al., 2008) and the effect of age in the present study.

The absence of a gilt age effect in the ovarian response to PG600 indicates that during the prepubertal phase, concurrent LH activity is necessary for continued follicular development. With PG600, this LH activity is supplied by the hCG component of the product. The results of the present study, where no effect of FSH pre-treatment on gilt estrous or ovulatory responses to eCG were evident also supports, albeit indirectly, the suggestion of an indispensable role for LH activity in follicular development. Indeed, development of follicles beyond 4 mm in diameter was not FSH dependent but seemed to be controlled primarily by LH pulses (Driancourt et al., 1995) and is coupled with a change in FSH and LH follicular receptors (Nakano et al., 1983; Liu et al., 1998, 2000). The improved response for induction of estrus of prepubertal gilts to larger doses of eCG (Dial et al., 1984; Britt et al., 1985) also indicates an indispensable role for LH activity in development of follicles to the pre-ovulatory stage. The eCG molecule does have some LH activity (Combarnous et al., 1984; Guthrie et al., 1990) and at larger doses it is possible that the LH activity of eCG becomes sufficient to allow development of large follicles. Indeed, LH (Huff and Esbenshade, 1992; Guthrie et al., 1990) and hCG (Bolamba et al., 1991; Driancourt et al., 1992) can stimulate follicular development, estrus and ovulation in pre-pubertal gilts if given in sufficient quantities, whereas treatment with FSH alone did not have any effect on the number of large follicles (Guthrie et al., 1988, 1990). In contrast, however, Bolamba and Sirard (2000) did note growth of large follicles when FSH was administered for 3 instead of 2 days (93–187 μg/kg BW 24 h interval), although this could be attributable to LH contamination of the FSH used. Additional evidence in support of the role of LH in follicular development was provided in the review of Evans and O'Doherty (2001).

In conclusion, the lesser ovulatory response to a relatively small dose of eCG compared to the combination of eCG and hCG, likely reflects an inability to stimulate growth of follicles, particularly at younger gilt ages. The absence of an effect of FSH pre-treatment on the response to eCG indicates that either the protocol used in the present study did not affect follicular growth or that there is an indispensable role for LH activity in final follicular development.

Acknowledgements

We gratefully acknowledge Bioniche Animal Health for the provision of Folltropin, UVESA for allowing access to the gilts, Evaristo Aguado for his expert assistance throughout the study, and Laboratorio de Tecnicas Instrumentals, University of Leon, for provision of laboratory facilities.

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CAPÍTULO 5: Effect of hCG Treatment on the Estrous and Ovulation Responses to FSH in Prepubertal Gilts

Published in Reproduction Domestic Animals, 2009; 44(3):432-434.

Reprod Dom Anim **44**, 432–434 (2009); doi: 10.1111/j.1439-0531.2008.01103.x
ISSN 0936-6768

Effect of hCG Treatment on the Oestrous and Ovulation Responses to FSH in Prepubertal Gilts

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Contents

To ensure sufficient numbers of pregnant females, particularly at hotter times of the year, hormonal induction of gilt oestrus may be necessary. However, the gilt oestrus and ovulation responses to gonadotrophin treatment have often proven unpredictable. The objective of this study was to examine possible reasons for this unpredictability. Prepubertal gilts (approximately 150 days of age, n = 63) were assigned to one of three treatments: injection of 300 IU hCG (n = 15); pre-treatment with 100 mg FSH in polyvinylpyrrolidinone administered as 2 × 50 mg injections 24 h apart, followed by 600 IU eCG at 24 h after the second FSH injection (n = 23); or FSH pre-treatment as above followed by 300 IU hCG at 24 h after the second FSH injection (n = 25). To facilitate oestrus detection, gilts were exposed to a mature boar for 15 min daily for 7 days. Blood samples were obtained on the day of eCG or hCG injection and again 10 days later and gilt ovulation responses determined based on elevated progesterone concentrations. The oestrus responses by 7 days were 6.7%, 17.5% and 64.0% for gilts treated with hCG, FSH + eCG and FSH + hCG, respectively ($p < 0.001$). The oestrous gilt receiving hCG alone and one oestrous FSH + hCG gilt did not ovulate, all other oestrous gilts ovulated. A further two anoestrous FSH + eCG-treated gilts ovulated. These data suggest that FSH pre-treatment facilitated the development of ovarian follicles to the point where they became responsive to hCG, but had little effect on the response to eCG.

Introduction

The output of the breeding herd is weaner pigs, and it has been suggested that the factor most affecting the predictability of weaner pig output is the meeting of breeding targets (Dial et al. 1996). The ability to meet the weekly breeding target requires a predictable supply of service-ready gilts, which may necessitate the hormonal induction of oestrus to ensure the availability of a sufficient number of breeding females. Gonadotrophic preparations used to stimulate oestrus in gilts are equine chorionic gonadotrophin (eCG) and combinations of eCG with human chorionic gonadotrophin (hCG). We have previously confirmed that, compared to treatment with 600 IU eCG alone, oestrus and ovulation responses of gilts were improved when injected with 400 IU eCG combined with 200 IU hCG (PG600®; Intervet International, Boxmeer, The Netherlands) (Manjarin et al. 2008a). However, responses to PG600 remained unpredictable, with 73% exhibiting oestrus, but only 64% exhibiting both oestrus and ovulating. We now speculate that the failure of overtly oestrous gilts to ovulate may be because of the 200 IU hCG in PG600 providing an inadequate duration of LH-like activity to complete follicular development. Interestingly, the serum half-life of hCG in humans was longer after subcutaneous than after intramuscular injection (Saal et al. 1991) and on one occasion an improved oestrus response of gilts to PG600 was noted after subcutaneous injection compared to that of intramuscular injection (Knox et al. 2000). This suggests a longer duration of gonadotrophic biological activity may be necessary for some gilts to both express oestrus and ovulate. However, prolonging the duration of gonadotrophin activity by simply increasing the initial dose of PG600 did not prove useful. The administration of 1.5 or 2.0 times the label dose of PG600 to gilts did increase the number of corpora lutea but also increased the numbers of gilts with follicular cysts as well as the numbers of cysts per gilt (Breen et al. 2006).

Several earlier studies have shown that LH (Huff and Esbenshade 1992) and hCG (Guthrie et al. 1990; Bolamba et al. 1991; Driancourt et al. 1992), in addition to their ovulatory and steroidogenic actions, can also stimulate follicular development. However, gilts with different ovarian status (number and size of follicles) at the time of hCG injection had different rates of ovulation (Bolamba et al. 1991), suggesting that hCG is unable to initiate growth of small follicles. In contrast, FSH seems to play a key role during initial follicle growth (Guthrie and Bolt 1990; Knox et al. 2003). Taking all together, we now hypothesize that pre-treatment of gilts for 2 days with FSH may advance follicular development to the point where the follicles will respond to hCG injection with onset of oestrus and ovulation.

Material and methods

These studies were performed during October to December 2007 at a commercial 1000-sow farrow-to-wean facility near Leon, Spain. Animals were cared for humanely in accordance with institutional animal care guidelines. To examine responses to different gonadotrophin preparations, 63 prepubertal Hypor gilts (approximately 150 days of age) were employed. Actual gilt ages were between 140 and 160 days but the farm recorded all gilts within this range as being 150 days. Gilts were housed at six to eight animals per pen and fed *ad libitum*, a wheat/soybean meal finisher diet formulated to provide 14 MJ DE/kg and 0.8% lysine.

At selection, gilts were assigned to receive an intramuscular (im) injection of 300 IU hCG (Veterin Corion®; Divasa, Barcelona, Spain) (n = 15), or 100 mg FSH (Folltropin®; Bioniche Animal Health, Bellville,

ON, Canada) mixed with polyvinylpyrrolidinone K-30 (PVP, Plasdone C-30, average mol wt. 58 000; ISP Technologies Inc., Wayne, NJ, USA), administered as 2×50 mg injections 24 h apart, followed by 600 IU eCG (Folligon®; Intervet International, Boxmeer, the Nether lands) at 24 h after the second FSH injection ($n = 23$), or 100 mg FSH as above, and then 300 IU hCG at 24 h after the second FSH injection ($n = 25$). The dose of eCG was chosen because of its known efficacy in weaned sows and it also reflects the total gonadotrophin content of PG600 (400 IU eCG plus 200 IU of hCG), a product of known efficacy in gilts. The dose of hCG was chosen to reflect the LH-like activity of PG600, i.e. 200 IU of hCG with the 400 IU eCG likely having 20–40% LH-like activity (Combarnous et al. 1984; Guthrie et al. 1990). The objective of administering FSH in PVP solution was to prolong the period of biological activity (Jackson et al. 2006). The PVP solution (30%) was prepared by dissolving 15 mg of PVP in 40 ml of distilled and deionized water and was stored at 4°C until use. For each injection, 3.125 ml PVP solution was mixed with 0.875 ml (50 mg) of FSH.

To facilitate detection of oestrus, gilts were subjected to direct exposure to a mature boar for 15 min daily from 1 to 7 days after hormone injection. Gilts were deemed to be oestrous if they adopted a rigid stance in the presence of the boar. To determine the ovulation responses to hormone treatment, blood samples were obtained from all gilts via jugular venipuncture at the time of hCG or eCG injection (day 0) and 10 days later. Serum was harvested and frozen (−20°C) until assayed for progesterone (P4) concentrations. Serum samples were assayed for P4 in a single assay using a commercial ELISA kit (Immulite®: Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Assay sensitivity and intra-assay coefficient of variation were 0.2 ng/ml and 8.1% respectively. All gilts employed in this study had non-detectable P4 concentrations on the day of injection. An elevation of P4 concentrations on day 10 to a minimum of 5 ng/ml was deemed to indicate an ovulation response to treatment (Althouse and Hixon 1999). Treatment differences for oestrus and ovulation response rates were examined by chi-squared test.

Results

As shown in Table 1, of the 25 gilts receiving FSH plus hCG, 16 (64%) exhibited oestrus by 7 days and all but one of these estrous gilts had serum P4 concentrations indicative of ovulation. None of the nine anoestrous FSH plus hCG-treated gilts ovulated. One gilt receiving hCG alone exhibited oestrus, but neither she nor any other gilt in this group ovulated. Of the 23 FSH plus eCG-treated gilts, four (17.4%) exhibited oestrus but only three of these ovulated. Of the 19 FSH plus eCG-treated gilts that remained anestrous, three had elevated serum P4 concentrations indicating ovulation.

Table 1. Effect of injecting hCG, or FSH followed by eCG, or FSH followed by hCG, into 150 days gilts on the incidence of oestrus by 7 days and ovulation as indicated by elevated progesterone (+P4), or non-ovulation (−P4)

	hCG[a]	FSH + eCG[b]	FSH + hCG[c]
No. of gilts	15	23	25
Gilts oestrus by 7 days (%)[d]	1 (6.66%)	4 (17.4)	16 (64.0)
Gilts ovulating (%)	0	6 (26.1)	15 (60.0)

[a]300 IU hCG.
[b]100 mg FSH in PVP as two injections 24 h apart then 600 IU eCG 24 h after last FSH.
[c]100 mg FSH in PVP as two injections 24 h apart then 300 IU hCG 24 h after last FSH.
[d]Effect of hormone preparation, $p < 0.001$ by chi-squared test.

Discussion

The results of this study support our hypothesis that pre-treating gilts with FSH will enhance the oestrus and ovulation responses to injection of hCG. Further, although we did not monitor ovarian follicular dynamics, we suggest that the better responses were because of an FSH-associated advancement of follicle maturity, presumably to the 4 mm stage that becomes responsive to LH-like activity. That prior FSH activity was necessary for expression of the hCG effect on oestrus and ovulation responses is also indicated by the lack of effect of hCG when not pre-treated with FSH.

The present results also support our previous findings (Manjarin et al. 2008a,b) and literature evidence (Gama et al. 2005; do Lago et al. 2005) that, in the absence of additional LH-like activity, 600 IU eCG, is relatively non-efficacious for induction of oestrus and ovulation in prepubertal gilts. The eCG molecule does have some LH-like activity (Combarnous et al. 1984; Guthrie et al. 1990) but apparently not enough to drive follicular development when only 600 IU is administered. Oestrus responses to eCG are increased when higher doses are administered (e.g. Dial et al. 1984; Bolamba et al. 1992), presumably because of the associated increase in LH-like activity. In a previous study, we noted no effect of FSH pre-treatment on the efficacy of eCG for oestrus induction, although in that study only 72 mg of FSH was injected and it was administered as six serial injections (Manjarin et al. 2008b). This study employed PVP to extend the biological activity of the 100 mg FSH but, again, FSH failed to affect the ovarian response to eCG. The FSH pre-treatment was effective for priming the ovary to respond to hCG, which does suggest an effect on follicle development. However, based on this data, the magnitude of the effect was not sufficient to allow the follicles to respond to eCG stimulation.

Driancourt et al. (1995) observed in gilts that follicle development to approximately 4 mm stage was dependant only on FSH while growth beyond this diameter appeared to be controlled by LH pulses. Indeed, granulosa cells of immature follicles contained exclusively FSH receptors, but these declined by the 4 mm stage and were absent in larger follicles (Liu et al. 1998, 2000). Interestingly, treatment *in vitro* with FSH promoted LHr mRNA expression (LaBarbera and Ryan 1981), which likely explains how the FSH pre-treatment was able to prime the ovary to respond to the hCG injection.

In conclusion, pre-treatment with FSH increased the ovulation response to hCG compared to that of hCG alone, supporting an indispensable role for FSH during early follicle maturation but a transition

to LH dependence. An improved understanding of this relationship may facilitate the development of improved protocols for hormonal induction of oestrus and ovulation.

Acknowledgements

We gratefully acknowledge Bioniche Animal Health for the provision of Folltropin, UVESA for allowing access to the gilts, Evaristo Aguado for his expert assistance throughout the study, Labatorio SYVA S.A., Leon, Spain for provision of PVP, and Laboratorio de Tecnicas Instrumentals, University of Leon, for provision of laboratory facilities.

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Submitted: 31 Jan 2008

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CAPÍTULO 6: Effect of hCG on Early Luteal Serum Progesterone Concentrations in PG600-treated Gilts

Published in Reproduction Domestic Animals, 2010; 45:555-557

Reproduction in Domestic Animals

Reprod Dom Anim **45**, 555–557 (2010); doi: 10.1111/j.1439-0531.2008.01299.x
ISSN 0936-6768

Short Communication

Effect of hCG on Early Luteal Serum Progesterone Concentrations in PG600-Treated Gilts

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Contents

Gilt oestrus and ovulation responses to injection of a combination of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) (PG600) can be unpredictable, possibly reflecting inadequate circulating LH activity. The objective of this study was to determine the effect of PG600 followed by supplemental hCG on gilt ovarian responses. In experiment 1, 212 Hypor gilts (160 day of age) housed on two farms in Spain received intramuscular (i.m.) injections of PG600 (n = 47), or PG600 with an additional 200 IU hCG injected either concurrently (hCG-0; n = 39), or at 24 h (hCG-24; n = 41) or 48 h (hCG-48; n = 45) after PG600. A further 40 gilts served as non-injected controls. Ovulation responses were determined on the basis of initial blood progesterone concentrations being < 1 ng/ml and achieving > 5 ng / ml 10 d after the PG600 injection. The incidence of ovulating gilts having progesterone concentrations > 30 ng/ml were recorded. During the study period, 10% of control gilts ovulated whereas 85–100% of hormone-treated gilts ovulated. There were no significant differences among hormone groups for proportions of gilts ovulating. The proportions of gilts having circulating progesterone concentrations > 30 ng/ml were increased ($p \leq 0.02$) in all hCG treated groups compared with the PG600 group. In experiment 2, a total of 76 Hypor gilts at either 150 or 200 days of age were injected with PG600 (n = 18), 400 IU eCG followed by 200 IU hCG 24 h later (n = 20), PG600 followed by 100 IU hCG 24 h later (n = 17), or 400 IU eCG followed by 300 IU hCG 24 h later (n = 21). Blood samples were obtained 10 days later for progesterone assay. There were no effects of treatment or age on incidence of ovulation, but fewer 150-day-old gilts treated with PG600 or 400 IU eCG followed by 200 IU hCG had progesterone concentrations > 30 ng / ml. We conclude that hCG treatment subsequent to PG600 treatment will generate a higher circulating progesterone concentration, although the effect is not evident in older, presumably peripubertal, gilts. The mechanism involved and implications for fertility remain to be determined.

Introduction

The output of the breeding herd is weaner pigs and it has been suggested that the factor most affecting the predictability of weaner pig output is the meeting of breeding targets (Dial et al. 1996). The ability to meet the weekly breeding target requires a predictable supply of service-ready gilts, which may necessitate the hormonal induction of oestrus to ensure availability of a sufficient number of breeding females. Gonadotrophic preparations used to stimulate oestrus in gilts are equine chorionic gonadotrophin (eCG) and combinations of eCG with human chorionic gonadotrophin (hCG). We have previously confirmed that, compared to treatment with 600 IU eCG alone, oestrus and ovulation responses of gilts were improved when injected with 400 IU eCG combined with 200 IU hCG (PG600®; Intervet International, Boxmeer, The Netherlands) (Manjarin et al. 2008a). However, responses to PG600 may still be improved as 73% exhibited oestrus and only 64% exhibited oestrus and ovulated. Similar oestrus response rates have been noted following PG600 treatment of primiparous sows at weaning, although rates of ovulation were not determined (Kirkwood et al. 1998).

The importance of LH activity for follicle development beyond 4 mm has been demonstrated previously (Driancourt et al. 1995), and we recently confirmed the biological significance of LH-like activity for oestrus and ovulation in gilts (Manjarin et al. 2008b). When gilts pre-treated with FSH subsequently received an injection of hCG, the oestrus and ovulation responses were significantly improved compared with the FSH-treated gilts that received eCG alone. Therefore, we now speculate that the failure of some oestrous gilts to ovulate after PG600 treatment may be because of the 200 IU hCG in PG600 providing an inadequate duration or intensity of LH-like activity to complete the development of ovarian follicles. In turn, our hypothesis is now that an increased duration of LH-like activity will enhance ovarian follicular development and associated rates of oestrus and ovulation. Indeed, the serum half-life of hCG in humans was longer after subcutaneous than after intramuscular injection (Saal et al. 1991) and on one occasion, an improved oestrus response to PG600 was noted after pre-pubertal gilts were injected subcutaneous compared with intramuscular suggesting a longer period of hormone exposure (Knox et al. 2000). The present study was undertaken to test the hypothesis that additional hCG will improve oestrus and ovulation rates to PG600 injection.

Material and Methods

These studies were performed on a 1000-sow farrow-to-wean facility near Leon, Spain (Farm 1) and on a 2500-sow farrow-to-wean facility near Avila, Spain (Farm 2) during December 2007 and January 2008 (experiment 1) and on farm 1 during July 2008 (experiment 2). For experiment 1, a total of 212 Hypor gilts (approximately

160 day of age) were employed to examine responses to different gonadotrophin preparations. Gilts were housed at 10–12 animals per pen and fed *ad libitum* a wheat/soybean meal finisher diet formulated to provide 14 MJ DE/kg and 0.8% lysine. At selection, gilts were assigned to receive an intramuscular injection (i.m.) of 400 IU eCG plus 200 IU hCG (PG600®; Intervet International, n = 26 and 21 for farms 1 and 2, respectively), or PG600 with 200 IU hCG (Chorulon®, Intervet International) injected IM either concurrently (hCG-0; n = 19 and 20), or after 24 h (hCG-24; n = 22 and 19), or 48 h from PG600 (hCG-48; n = 23 and 22). An additional 20 gilts per farm served as non-injected controls. The hCG dose was chosen on the basis of the known efficacy of the 200 IU hCG dose within the PG600 combination.

In experiment 2, a total of 76 Hypor gilts at either 150 or 200 days of age were injected with PG600 (n = 18), 400 IU eCG followed by 200 IU hCG 24 h later (n = 20), PG600 followed by 100 IU hCG 24 h later (n = 17), or 400 IU eCG followed by 300 IU hCG 24 h later (n = 21).

To determine the ovulation responses to hormone treatment in experiment 1, blood samples were obtained from all gilts by jugular venipuncture at the time of PG600 injection and again 10 days later. Serum was harvested and frozen (−20°C) until assayed for progesterone concentrations in a single assay using a commercial ELISA kit (Immulite®, Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Assay sensitivity and intra-assay coefficient of variation were 0.2 ng/ml and 8.1%, respectively. An elevation in progesterone concentrations from <1 ng/ml at PG600 injection to a minimum of 5 ng/ml on day 10 was deemed to indicate an ovulation response to treatment (Althouse and Hixon 1999). For experiment 2, gilts were bled only once, 10 days after PG600 or eCG injections, and serum assayed for progesterone as described above. Assay characteristics were unchanged.

Treatment differences for ovulation response rates, and proportions of ovulating gilts having serum progesterone concentrations >30 ng/mL, were examined by the CATMOD procedure in SAS version 9.1 (Statistical Analysis Systems, Cary, NC, USA). For experiment 1, initial analysis indicated no effect of farm or farm x treatment interaction, so data were pooled across farm.

Results

For experiment 1, all gilts had non-detectable progesterone concentrations on the day of PG600 injection. During the study period four control gilts ovulated whereas 85–100% of hormone-treated gilts ovulated. There were no significant differences among hormone-treated groups for proportions of gilts ovulating. However, the proportion of gilts having circulating progesterone concentrations >30 ng/mL were increased ($p \leq 0.02$) in all hCG treated groups compared with the PG600 group (Table 1).

In experiment 2, there were no effects of treatment or age on incidence of ovulation. Compared with 150-day-old gilts receiving PG600 followed by 100 IU hCG, fewer ($p < 0.02$) 150-day-old gilts treated with PG600 or 400 IU eCG followed by 200 IU hCG had progesterone concentrations >30 ng/ml, with the incidence being intermediate for gilts receiving eCG followed by 300 IU hCG (Table 2). There were no treatment differences for incidence of higher progesterone concentrations among the 200-day-old gilts (Table 2).

Table 1. Effect of PG600 or PG600 followed by hCG at 0, 24, or 48 h on ovulation responses of pre-pubertal gilts

	Control	PG600	hCG 0 h[1]	hCG 24 h[1]	hCG 48 h[1]
No. of gilts	40	47	39	41	45
Gilts ovulating[2,3]	4 (10)	40 (85.1)	36 (92.3)	41 (100)	43 (95.6)
P4 > 30 ng/mL[3,4]	0	19 (47.5)[a]	33 (91.7)[b]	38 (92.7)[b]	32 (74.4)[b]

[a,b]Means followed by different letters are different, $p \leq 0.02$.
[1]Hours are the interval between injections of PG600 and 200 IU hCG.
[2]Indicated by circulating progesterone concentrations of at least 5 ng/ml.
[3]Data in parenthesis are percentages.
[4]No. of gilts having progesterone concentrations >30 ng/ml; Differences examined with Chi square. The percentage is based on ovulating gilts.

Discussion

These studies assessed the possible beneficial effects of supplemental LH-like activity at different stages (0, 24 and 48 h post-follicular phase induction) of a follicular phase induced by PG600. The results suggest that such an approach may increase the proportions of gilts expressing high progesterone concentrations following ovulation, although results from experiment 2 suggests the effect is age-dependant, being evident only at younger ages. Although speculative, these data indicating an age effect support the suggestion that the failure of some gilts to exhibit oestrus and ovulate in response to PG600 is because of the inadequate endogenous LH support for follicular growth. As the gilt population ages and matures, endogenous LH support becomes

Table 2. Influence of gilt age on the effect of PG600 or PG600 followed by hCG on ovulation responses and serum progesterone concentrations in gilts

	PG600	eCG + hCG-200[1]	PG600 + hCG-100[2]	eCG + hCG-300[3]
150 day				
No. of gilts	9	10	9	11
No. of ovulating[4,5]	8 (88.9)	10 (100)	9 (100)	10 (90.9)
No. of P4 > 30 ng/ml[5]	2 (25.0)[b]	3 (30.0)[b]	8 (88.9)[a]	5 (50.0)[ab]
200 day				
No. of gilts	9	10	8	10
No. of ovulating[4,5]	9 (100)	10 (100)	8 (100)	10 (100)
No. of P4 > 30 ng/ml[5]	8 (88.9)	10 (100)	8 (100)	8 (80.0)

[a,b]Means followed by different letters are different, $p < 0.02$.
[1]400 IU eCG followed after 24 h with 200 IU hCG.
[2]PG600 followed after 24 h with 100 IU hCG.
[3]400 IU eCG followed after 24 h with 300 IU hCG.
[4]Indicated by circulating progesterone concentrations of at least 5 ng/ml.
[5]Data in parenthesis are percentages. For P4 > 30 ng/ml, it is based on ovulating gilts and differences examined using Fisher's exact test.

adequate and so a response to supplemental hCG is not evident.

The responses observed do support our current understanding of the control of terminal follicular growth in swine, as previous workers have documented improved oestrus and ovulatory responses when high doses of hCG were administered to gilts (Bolamba et al. 1991; Driancourt et al. 1992). The significant effect of hCG supplementation on circulating progesterone concentrations may be a consequence of either an increase in numbers of corpora lutea caused by reduced follicular atresia throughout the follicular phase, or to an increased progesterone output by individual corpora lutea. However, because we did not monitor ovarian dynamics by ultrasound, the origin of the higher circulating progesterone remains speculative. The present results do support literature evidence indicating an important role for LH during the final phase of follicular development (Evans and O'Doherty 2001).

In contrast to other farm animal species, terminal follicular growth in swine proved to be strongly LH dependent (Driancourt et al. 1995). Further, an early development of LH receptors in swine ovarian granulose cells *in vitro* has been demonstrated (Liu et al. 1998, 2000). These observations, together with the increasing LH concentrations throughout the follicular phase may explain the effects of supplementation with hCG during the early follicular phase. The more clear cut effects of hCG supplementation noted at 0 and 24 h post PG600 compared with that at 48 h after PG600 in experiment 1 is puzzling. It was initially believed that administration of LH support (via hCG) during the mid follicular phase would expose the growing/maturing follicles to a very wide time window of LH-like activity and therefore optimise results. This timing did not prove to be superior to the other groups treated earlier during the follicular phase. One possible explanation might be that, in the early dominance phase, follicles may rely heavily on LH for their continued growth, survival, and maturation. In contrast, in the mid follicular phase, decreased expression of IGFBP2 will result in higher bio-availability of IGF. The IGFs will then synergise with existing LH concentrations to support final follicle development and maturation (Lucy et al. 2001).

We conclude that hCG treatment subsequent to PG600 treatment will generate a higher circulating progesterone concentration, although the effect is not evident in older, presumably peripubertal, gilts. The mechanism involved and implications for fertility remain to be determined.

Acknowledgements

We gratefully acknowledge Intervet/Schering Plough Animal Health, Boxmeer, the Netherlands for financial support and the provision of PG600 and Chorulon. We thank GPA for allowing access to the gilts, Evaristo Aguado and Pilar Fernandez for their expert assistance throughout the study, and Laboratorio de Tecnicas Instrumentals, University of Leon, for provision of laboratory facilities.

Author contributions

Manjarin, Dominguez, Alegre and Kirkwood designed the study and were involved in data interpretation and analysis. Driancourt was involved in data analysis and interpretation. Manjarin and Castro managed the study. The manuscript was produced by Kirkwood, Driancourt and Manjarin.

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Submitted: 26 Sep 2008

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CAPÍTULO 7: Effect of additional hCG on follicular growth and ovulation in PG600-treated gilts

Submitted to Journal of Animal Science, November 2013

Running head: Hormonal manipulation of ovulation in gilts

Effect of additional hCG on follicular growth and ovulation in PG600-treated gilts

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ABSTRACT: We have previously shown that injection of 200 IU human chorionic gonadotrophin (hCG) at 24 or 48 h after PG600 (400IU equine chorionic gonadotrophin + 200IU hCG) injection markedly increased early luteal blood progesterone concentrations in gilts. The objective of the present study was to determine the effect of supplemental hCG on ovarian responses of gilts. Forty nine prepubertal gilts received an injection of PG600 on d 0, and then were allotted by weight to receive 100 IU hCG at 24 h (n = 20; hCG-100), 200 IU hCG at 24 h (n = 10; hCG-200), or served as control (n = 19; hCG-0). Twenty six gilts were slaughtered 10 d after PG600 and their ovaries recovered for determination of numbers of corpora lutea (ovulation rate) and numbers of follicular cysts. Ovulatory response in the remaining gilts was determined by transrectal real-time ultrasonography at initial gonadotrophin injection and at d 5 and 10 post treatment. Reproductive parameters were analyzed by logistic regression and ANOVA, and and linear and quadratic trends were computed using orthogonal contrasts. Follicle sizes were ≤ 3 mm prior treatment. Increasing levels of hCG did not improve the ovulatory response in gilts ($P = 0.14$), but when the hCG-0 group was compared to hCG-100 and 200 together, the number of ovulating gilts tended to increase ($P = 0.09$) in response to supplemental hCG. Number of corpora lutea increased (Linear, $P = 0.027$) with hCG supplementation, and it was higher in hCG-200 gilts compared to hCG-100 and hCG-0 ($P = 0.028$ and 0.027, respectively). The frequency of follicle cysts (>12 mm) in gilts increased with hCG dose (Linear, $P = 0.0014$); it was higher in hCG-100 compared to hCG-0 ($P = 0.032$), and it further increased in hCG-200 group ($P = 0.04$). The number of cysts per gilt also increased (Quadratic, $P = 0.05$); compared to hCG-0, it was higher in both hCG-100 and hCG-200 ($P = 0.006$ and 0.001, respectively). All the ovarian cysts were classified as follicular. We conclude that, compared to injection of PG600 alone, supplemental hCG tends to increase the ovulatory response and increases the number of corpora lutea in gilts, but its use is associated to cyst development in a dose dependent

manner. A better understanding of the effect of LH on follicular growth may facilitate the development of improved protocols for hormonal induction of estrus and ovulation in prepubertal gilts.

Keywords: cyst, estrus, follicle, gilt, PG600, superovulation

INTRODUCTION

Predictability of weaner pig output is largely dependent on breeding sufficient females each week (Dial et al., 1996), and the meeting of these weekly breeding targets requires a predictable supply of service-ready gilts, which may necessitate the hormonal induction of estrus to ensure their availability. The gonadotrophin preparation most often used to stimulate estrus in gilts is PG600, which is a combination of 400 IU equine chorionic gonadotrophin (eCG) and 200 IU human chorionic gonadotrophin (hCG). Estrus responses of gilts to PG600 injection are variable, with reported response rates of 37 or 64% (Tilton et al., 1995), 52 or 76% (Knox et al., 2000), and 73 or 78% (Kirkwood, 1999; Manjarín et al., 2009a). The underlying cause of these variable responses remain undetermined, although it is likely that degree of ovarian maturation at the time of treatment and level of endogenous gonadotrophic support of ovarian function are involved.

In swine, the primary driver of follicle development beyond 4 mm is LH (Driancourt et al., 1995). Confirming this, we recently observed that when gilts pre-treated with FSH received an injection of hCG the estrus responses were improved compared to FSH-treated gilts that received eCG (Manjarín et al., 2009b). Therefore, we speculated that failure of gilts to respond to PG600 treatment may be due to the 200 IU hCG in PG600 providing an inadequate duration of LH-like activity, and as such, increasing the duration of LH-like activity would enhance ovarian follicular development and associated rates of ovulation.

When supplemental hCG was administered to gilts following PG600 treatment, circulating progesterone concentrations were markedly increased, although the etiology was not determined (Manjarín et al., 2010a). The present studies were undertaken to further examine gilt responses to supplemental hCG and to test the hypothesis that additional hCG will increase the number of follicles ovulating in response to PG600 treatment in gilts.

MATERIAL AND METHODS

Animals and treatments

These studies were performed on a 800-sow farrow-to-finish facility near Guelph, Ontario, Canada. Animals were cared for in accordance with local animal care guidelines. To examine gilt responses to different gonadotrophin combinations, a total of 49 prepubertal Yorkshire × Landrace gilts (107 ± 7.5 kg) were used in 2 consecutive replicates. Gilts were housed at 8 or 10 animals per pen (approximately 1.6 $m^2 \cdot gilt^{-1}$) and fed ad libitum a corn-soybean meal finisher diet formulated to provide 13.7 MJ $ME \cdot kg^{-1}$, 14.0% crude protein, and 0.79% lysine. All gilts received an intramuscular (IM) injection of PG600 (Intervet/MSD, Boxmeer, The Netherlands) on d 0, and then were allotted by weight to 3 groups to receive a different dose of supplemental hCG (Chorulon®, Intervet/MSD) 24 h later. Group 1 gilts (n = 19; hCG-0) did not receive supplemental hCG and served as controls, group 2 gilts (n = 20; hCG-100) received 100 IU hCG and group 3 gilts (n = 10; hCG-200) received 200 IU hCG. At d 10 after PG600, 26 gilts (n = 8, 8, and 10 for hCG-0, hCG-100, and hCG-200, respectively) were slaughtered and their ovaries recovered for determination of numbers of corpora lutea (ovulation rate) and numbers of follicular cysts (> 12 mm). Treatment with hCG-200 was discontinued due to the high number of ovarian cysts observed in gilts. Ovulatory response in the remaining gilts was determined by transrectal real-time

ultrasonography (RTU) using an Aloka 500 with a 7.5 MHz linear array transducer at the time of PG600 injection, and 5 and 10 d later.

Statistical analysis

Differences in the incidence of ovulation and cyst development between groups were assessed by logistic regression analysis using a generalized linear mixed model in SAS 9.2 (PROC GLIMMIX; SAS Institute Inc., Cary, NC), assuming a binary distribution of the response variables. The linear model included treatment as fixed effect, block as random effect and initial gilt weight as covariate. Differences on numbers of corpora lutea and cysts were analyzed by a one-way ANOVA using a linear mixed model in SAS (PROC MIXED), that included the same parameters mentioned above. Normality of the residuals, presence of outliers and homogeneity of the variance was assessed by PROC UNIVARIATE (SAS) using the Shapiro-Wilk test, Q-Q-plot, externally studentized residuals and Levene´s test. When necessary, data were power transformed by a parameter φ whose optimal value was estimated using the maximum likelihood (ML) method (Piephø, 2009). Preplanned comparisons between treatments and linear and quadratic trends were computed using orthogonal contrasts in SAS, and *p*-values calculated using Student's t tests. Data is presented as probabilities and least square means ± SE. Significant effects were considered at $P < 0.05$.

RESULTS

At the start of the study, follicle sizes were ≤ 3 mm in all gilts. Increasing levels of hCG did not improve the ovulatory responses of gilts, but when the hCG-0 group was compared to hCG-100 and 200 together, the number of ovulating gilts tended to increase in response to supplemental hCG ($P = 0.09$; Table 1). Number of corpora lutea increased (Linear, $P = 0.027$) with hCG supplementation, and it was higher in hCG-200 gilts compared

110 to hCG-100 and hCG-0 (P = 0.028 and 0.027, respectively). The frequency of follicle cysts
111 (> 12 mm) in gilts increased with hCG dose (Linear, P = 0.0014); it was higher in hCG-100
112 compared to hCG-0 (P = 0.032), and it further increased in hCG-200 group (P = 0.04). The
113 number of cysts per gilt also increased (Quadratic, P = 0.05); compared to hCG-0, it was
114 higher in both hCG-100 and hCG-200 (P = 0.006 and 0.001, respectively). All the ovarian
115 cysts were classified as follicular.

116

117 **DISCUSSION**

118 This study assessed the effects of supplemental LH-like activity during a follicular
119 phase induced by PG600. Results from this experiment indicate that the number of corpora
120 lutea on ovaries of gilts increased when PG600 was supplemented with hCG 24 h later and
121 this increase was hCG dose-dependent. As such, these results support our hypothesis that
122 additional hCG increases the number of follicles ovulating in response to PG600 treatment in
123 gilts, and agrees with our previous findings that administration of supplemental hCG
124 significantly increases circulating progesterone levels in prepubertal gilts, likely due to a
125 higher number of luteal bodies (Manjarin et al., 2010a). The increase in corpora lutea could
126 be an androgen-dependant effect since increased follicular androgen synthesis has been
127 associated with hCG-induced superovulation in hamsters (Greenwald, 1993) and, in follicular
128 phase gilts, exogenous testosterone was associated with larger numbers of preovulatory
129 follicles and corpora lutea, although blastocyst production was negatively affected at higher
130 doses (Cardenas and Pope, 1994, 1997).

131

132 In addition to greater luteal body formation, the highest dose of supplemental hCG
133 (hCG-200) also induced multiple follicular cysts in most gilts, while at the lower hCG dose
134 (hCG-100) there was less incidence of gilts with cysts, and only 1 to 2 cysts per animal. A

similar relationship between gonadotrophin levels and incidence of cysts was also observed by Breen et al. (2005), with 1200 IU of PG600 inducing more cysts in 180 d gilts than 900 or 600 IU. In the present study, a negative effect of excess hCG at the ovarian level is suggested by the hCG-200 gilts having both many follicular cysts and corpora lutea, indicating that an ovulatory signal was received but not acted on by all follicles. We have previously shown an effect of age and body weight at eCG or PG600 treatment on the incidence of estrus and ovulation, with more of the gilts weighing > 90 kg or 180 d old exhibiting estrus and ovulation than their lighter and younger counter-parts (Manjarin et al., 2009a, c, 2010a). These studies suggests that as the gilt population ages and mature, endogenous LH support may become adequate to complete follicular development in response to PG600, and supplemental hCG is not longer needed. Given that the average gilt weight in the current study was 106 kg, it is possible that some of the gilts were mature enough to respond to PG600 alone, so administration of supplemental hCG may have caused an excess of LH activity leading to cyst development. A detrimental effect on fertility associated to sow´s age upon hCG supplementation was found in a previous study, when injection of 200IU of hCG 24 h after PG600 decreased farrowing rates in parity ≥ 3 sows, but not in younger counter-parts (Manjarín et al., 2010b).

It is possible that an excess of hCG down-regulated LH receptors in some follicles, as has been shown to occur in rat granulosa cells (LaPolt et al., 1990), which could have the effect of inhibiting their response to the ovulatory LH surge causing the development of ovarian cysts. Alternatively, it is possible that an excess of hCG, in presence of remaining FSH-like activity from the PG600, induced the selection of accessory waves of antral follicles in some of the gilts, and these were not ready to ovulate in response to the LH surge. Previous studies in pigs have shown that during the onset of the follicular phase, growth of antral

follicles beyond 3-4 mm becomes gradually dependent on LH activity, while smaller follicles, lacking LH receptors, undergo atresia (Driancourt et al., 1995; Liu et al., 1998; 2000). Transrectal ultrasonography indicated the presence of 3 mm follicles in some gilts prior gonadotrophin treatment, that were potentially responsive to the LH-like activity of the PG600, as shown by 63% ovulatory response in the hCG-0 group. Administration of 200 IU of hCG to these animals may have selected additional follicles at the time of the injection but also in consecutive days, that at a growth rate of 1 $mm \cdot d^{-1}$ (Morbeck et al., 1992), would not be ready to ovulate upon the LH peak, becoming follicular cysts. Although speculative, 100 IU of hCG may have selected additional follicles only around the time of the injection, which then could be less likely to develop cysts in those animals already responsive to PG600, while improving follicular growth within the 37% pool of unresponsive gilts. It is possible that a smaller dose of hCG (i.e. 20-50 IU) injected 24h after PG600 may still be able to select follicles in most of the prepubertal gilts without inducing cyst formation. Alternatively, the administration of eCG alone may be able to synchronize the growth of most follicles to the 4 mm window, when they would become responsive to the LH-like activity of the hCG. According to this, administration of 400IU eCG followed by 200 IU hCG 24 h apart had a 100% ovulatory rate, while very few 150 d animals had progesterone levels above 30 $ng \cdot \mu L^{-1}$, suggesting a normal ovulatory response (Manjarín et al., 2010a).

Taken together, these studies indicate that the LH/FSH ratio of PG600 was optimized to induce ovulation in gilts without causing follicular cyst development, although about 35% of the animals failed to respond to the treatment. We conclude that hCG treatment subsequent to PG600 induces a higher ovulatory response in gilts compared to injection of PG600 alone, but its use is associated to cyst development in a dose-dependent manner. A better

184 understanding of the effect of LH activity on follicular growth may facilitate the development
185 of improved protocols for hormonal induction of estrus and ovulation in prepubertal gilts.
186

187 **LITERATURE CITED**

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TABLES

Table 1: Effect of human chorionic gonadotrophin (hCG) supplementation on estrus and ovulatory responses in PG600-treated gilts

Item	hCG-0[1]	hCG-100[1]	hCG-200[1]	*P*-value[2]	
				L	Q
No. gilts	19	20	10	--	--
Weight, kg[3]	109 ± 2.6	107 ± 2.6	106 ± 2.3	--	--
Ovulation, %[4,5]	12 (63.1)	16 (80)	10 (100)	0.14	0.98
No. corpora lutea	19.75 ± 4.3^{d}	23.62 ± 4.3^{d}	38.4 ± 3.9^{e}	0.02	0.24
No. gilts with follicular cysts, %[6]	0^{a}	8 (40)^{b}	8 (80)^{c}	0.0014	0.71
No. cysts per gilt	0^{a}	1.25 ± 3.3^{b,d}	17.5 ± 2.3^{e}	0.006	0.05

[1]0,100 and 200 refer to dose (IU) of hCG (Chorulon®, Intervet/MSD, Boxmeer, The Netherlands) administered 24 h after PG600 (Intervet/MSD) injection

[2]*P*-value for linear and quadratic trends. L=linear, Q=quadratic

[3]Means±SE

[4]Determined at slaughter or by transrectal ultrasonography on d 10 after PG600

[5]Data in parenthesis are percentages

[6]Follicles >12 mm

[a-e]Means lacking a common superscript differ at: [a,b,c]$P \leq 0.05$ [d,e]$P \leq 0.01$

CAPÍTULO 8: Resumen de resultados, discusión y conclusiones

8.1 Resumen global de resultados

Cinco experimentos fueron desarrollados para examinar las razones de la impredictibilidad en las respuestas estral y ovulatoria en cerdas nulíparas asociada a la administración de las hormonas eCG y hCG. En el primer experimento, 109 cerdas prepúberes de 153 días de edad fueron distribuidas en dos grupos basados en el peso de los animales (75-90 kg, n=55 and 91- 110 kg, n=55), e inyectadas aleatoriamente con uno de tres posibles tratamientos hormonales: 1) inyección de 400 IU de eCG y 200 IU de hCG (PG600), 2) inyección de 600 IU de eCG sola, y 3) no inyección de gonadotropinas (grupo control). La respuesta estral fue caracterizada mediante la expresión del reflejo de inmovilidad inducido por la exposición directa a un macho adulto durante 15 min entre los días 2 y 7 posteriores a la inyección de hormonas. La respuesta ovulatoria fue valorada mediante el análisis de la concentración de progesterona (P4) en muestras de sangre obtenidas de la vena yugular en los d 0, 3 y 10 respecto a la inyección hormonal. Una elevación en la concentración de P4 $\geq$1 ng/mL entre los d 0 y 3 posteriores al tratamiento fue definida como ovulación prematura, mientras que niveles de P4 < 1 ng/mL en los d 0 y 3, seguidos de P4 $\geq$ 5 ng/ml en el d 10 fue considerado como una respuesta ovulatoria normal. El crecimiento folicular previo a la inyección de gonadotropinas fue determinado mediante ultrasonografía ovárica transrectal. El diámetro medio del tamaño de los folículos previo al tratamiento hormonal fue de 2.4 mm en el conjunto de la población analizada. Ninguna de las hembras del grupo control mostró celo u ovulación. En comparación con la administración de eCG sola, el tratamiento con PG600 resultó en un mayor porcentaje de hembras con síntomas de celo, asi como con niveles posovulatorios de P4 en el d 10. Ninguna de las cerdas tratadas con PG600 que no tuvo síntomas de celo presentó niveles elevados de P4 en el día 10. Finalmente, tanto la tasa de

celo como de ovulación estuvieron positivamente relacionadas con el peso de las cerdas prepúberes, siendo más altas en las cerdas con mayor peso al momento del tratamiento.

En el segundo experimento, 149 cerdas prepúberes fueron seleccionadas a los 150 o 180 d de edad y distribuidas en 4 grupos con tratamientos diferentes: 1) PG600, 2) 600 IU de eCG, 3) 72 mg de FSH administrado en 6 inyecciones consecutivas a intervalos de 12 h, seguido de una inyección de 600 IU de eCG 12 h después de la última administración de FSH, y 4) no inyección hormonal (grupo control). Las tasas de celo y de la ovulación fueron medidas y analizadas usando los mismos procedimientos que en el experimento anterior, con la diferencia de que el peso de los animales fue sustituido por la edad como efecto fijo en el modelo mixto linear. Comparado con la administración de PG600, tanto la administración de eCG sola como el tratamiento con FSH previo a la administración de eCG disminuyeron el porcentaje de hembras con niveles posovulatorios de P4, mientras que no se detectaron diferencias entre los grupos tratados con eCG y FSH + eCG. Además, la tasa de celos estuvo positivamente relacionada con la edad de los animales en el momento del tratamiento, siendo más elevada en los animales de mayor edad.

En el tercer experimento, 63 cerdas prepúberes (150 d) fueron asignadas a 3 tratamientos diferentes: 1) 300 IU de hCG, 2) 100 mg de FSH administrado en 2 inyecciones consecutivas a intervalos de 24 h, seguido de 300 IU de hCG 24 h después de la última administración de FSH, y 3) 100 mg de FSH administrado en 2 inyecciones consecutivas a intervalos de 24 h, seguido de una inyección de 600 IU de eCG 24 h después de la última administración de FSH. Las tasas de celo y de la ovulación fueron medidas y analizadas usando los mismos procedimientos que en el primer experimento. Las respuestas estral y

ovulatoria fueron mínimas en respuesta al tratamiento con hCG sola, y mejoraron con la administración de FSH seguida de

eCG. Cuando hCG fue administrada posteriormente a la FSH, las respuestas estral y ovulatoria fueron comparables a las inducidas por la PG600. En el cuarto experimento se realizaron dos experimentos diferentes. En el primer experimento, 212 cerdas prepúberes (160 d) fueron distribuidas en 5 tratamientos hormonales diferentes: 1) PG600, 2) PG600 y 200 IU de hCG administrados conjuntamente, 3) PG600 seguido de 200 IU de hCG administrado a las 24 h, 4) PG600 seguido de 200 IU de hCG administrado a las 48 h, y 5) no inyección de gonadotropinas (grupo control). La detección de celos y ovulación fue medida usando los mismos procedimientos que en el primer experimento. Comparado con la administración de PG600 sola, la administración de PG600 y hCG de forma conjunta o consecutiva no resultó en un aumento significativo del porcentaje de hembras en celo o con niveles posovulatorios de P4. Sin embargo, el porcentaje de hembras con niveles circulantes de P4 $\geq$ 30 ng/mL fue superior en todos los grupos que recibieron hCG suplementaria, en comparación con la administración de PG600 sola. En el segundo experimento 76 cerdas prepúberes fueron seleccionadas a los 150 o 200 días de edad y distribuidas en 4 tratamientos hormonales diferentes: 1) PG600, 2) PG600 seguido de 100 IU de hCG administrada a las 24 h, 3) 400 IU de eCG, seguido de 200 IU de hCG administrado a las 24 h, 3) 400 IU de eCG, seguido de 300 IU de hCG administrado a las 24 h. Comparado con la administración de PG600 sola, la administración de PG600 seguido de hCG, o de eCG seguido de hCG, no resultó en un mayor porcentaje de hembras en celo o con niveles posovulatorios de P4. Sin embargo, el porcentaje de hembras con niveles circulantes de P $\geq$ 30 ng/mL fue superior en el grupo de hembras de 150 d que recibió PG600 seguido de hCG a las 24 h, aunque este efecto no fue evidente en el grupo de cerdas nulíparas de 200 d de edad.

En el quinto experimento, 49 cerdas prepúberes fueron seleccionadas a los 160 días de edad y distribuidas en 3 tratamientos diferentes: 1) PG600 (control), 2) PG600 seguido de 100 IU de hCG administrada a las 24 h, y 3) PG600 seguido de 200 IU de hCG administrada a las 24 h. El crecimiento folicular previo a la inyección de gonadotropinas fue determinado mediante ultrasonografía ovárica. Para valorar la tasa de ovulación, veintiséis hembras fueron sacrificadas 10 días después de la inyección de PG600, y el número de cuerpos lúteos y quistes ováricos cuantificado. La ovulación en el resto de los animales se midió mediante ultrasonografía ovárica en el día 10 posterior a la PG600. En comparación con PG600, la administración de PG600 + hCG (grupos 2 y 3 en conjunto) resulto en un mayor porcentaje de hembras con niveles posovulatorios de progesterona. Además, se detecto un incremento lineal en el número de cuerpos lúteos en relación con la dosis de hCG administrada, siendo superior en el grupo que recibió 200IU comparado con la inyección de 100IU o con el grupo control. Igualmente, tanto el porcentaje de hembras con quistes ováricos como el numero de quistes por hembra aumentaron linealmente con la dosis de hCG, siendo superior en el grupo que recibió 200IU comparado con la inyección de 100IU, y este ultimo superior al grupo control. Todos los quistes ováricos fueron clasificados de naturaleza folicular.

8.2 Discusión

Los resultados del primer experimento indican que cuando los ovarios de las cerdas prepúberes contienen folículos de menos de 3 mm de diámetro, la falta de respuesta ovulatoria en el 30% de los animales tratados con PG600 no está asociada con una prematura ovulación o luteinización de los folículos inducida por la hormona hCG. De hecho, la escasa tasa de celos registrada en respuesta a la inyección de eCG comparado con la PG600, sugiere que la hCG tiene un papel fundamental en el desarrollo folicular y la inducción del celo. Cuatro de las hembras tratadas con PG600 que expresaron celo no tenían niveles elevados de progesterona en sangre. Es posible que en estas cerdas se produjera el crecimiento folicular necesario para causar un aumento en los niveles de estrógenos con el consiguiente celo, pero insuficiente para desencadenar el pico de LH. Sin embargo, en ausencia de la medición de los niveles de LH en sangre, esta hipótesis es especulativa.

La escasa respuesta estral y ovulatoria inducida por la administración de eCG consecutiva a la FSH en cerdas prepúberes (segundo experimento), sugiere que la falta de actividad de la eCG no es debida a su incapacidad para iniciar el crecimiento de los folículos de pequeño tamaño, como se había especulado en un principio. Aunque el desarrollo folicular en respuesta al pretratamiento con FSH no fue monitorizado en este estudio, el protocolo de administración de la FSH fue similar a estudios anteriores, en los que si se demostró un crecimiento de los folículos hasta los 4 mm de diámetro (Guthrie et al, 1990; Bolamba et al., 1996). En este sentido, en el tercer experimento de la tesis no solo se aumento la dosis de FSH, sino también se mezcló con un polímero de liberación retardada (polivinilpirrolidona, PVP) para aumentar su vida media en sangre. Sin embargo la inyección de eCG consecutiva a la FSH+PVP siguió sin traducirse en un incremento del número de ovulaciones.

El tratamiento con FSH previo a la administración de hCG (tercer experimento), aumentó la respuesta ovulatoria en comparación con la administración de hCG sola, lo que sugiere que la hormona FSH tiene un papel fundamental durante las primeras fases del desarrollo folicular. Además, la mayor respuesta ovulatoria obtenida con la administración de FSH seguida de hCG en vez de eCG sugiere un indispensable papel de la hormona hCG (con actividad LH) en el crecimiento folicular, una vez que los folículos han alcanzado un cierto desarrollo. Estos resultados proveen una explicación a la mejora en la inducción del celo y la ovulación en respuesta a altas dosis de eCG (700-1000 IU) observada en experimentos anteriores (Guthrie, 1977; Dial et al., 1984; Britt et al., 1986; Esbenshade, 1987; Flores et al., 1989; Bolamba, 1992), asi como las tasas de celo entre el 25 % y el 52 % cuando se inyectaron solamente entre 300 y 600 IU de eCG (Britt et al., 1985; do Lago et al., 2005; Gama et al., 2005). La hormona eCG, además de actividad FSH, presenta cierta actividad LH (Combarnous et al., 1984; Guthrie et al., 1990), de forma que administrada en mayores dosis podría aportar una cantidad de LH suficiente para permitir el desarrollo de los folículos hasta la ovulación. La necesidad de la actividad LH en el desarrollo folicular también explicaría el aumento en el porcentaje de celos y ovulaciones en respuesta al tratamiento con eCG en las cerdas de mayor peso y edad, observado en experimentos 1 y 2, respectivamente. A medida que las cerdas crecen se produce una maduración fisiológica del eje hipotálamo-hipófisis-ovario, que se traduce en un aumento de los niveles endógenos de la gonadotropina LH, que podría complementar la falta de actividad LH de la gonadotropina eCG para completar el desarrollo folicular previo a la ovulación.

Aunque en el tercer experimento no se monitorizó la dinámica folicular, los resultados observados nos llevaron a especular que la mejora en la respuesta ovulatoria inducida por la administración consecutiva de FSH y hCG, podría ser debida a un control bifásico del

crecimiento folicular por parte de las gonadotropinas hipofisarias. En este sentido, Driancourt et al., (1995) observó que, en cerdas nulíparas, el crecimiento folicular hasta un diámetro cercano a los 4 mm era dependiente de la FSH, mientras que el crecimiento más allá de este tamaño parecía ser controlado por pulsos de LH. Este cambio en la dependencia gonadotrópica de los folículos parece estar acoplado a un cambio en la expresión de los receptores para la LH y FSH, con una disminución del ARNm que codifica para el receptor de la FSH y un aumento del de la LH (Nakano et al.,, 1983; Liu et al.,1998, 2000). Interesantemente, el tratamiento de folículos con FSH aumentó la expresión del receptor de LH a nivel del ARNm *in vitro* (LaBarbera and Ryan, 1981), lo que podría explicar cómo el pretratamiento con FSH aumento la respuesta ovárica a la hCG en nuestro tercer experimento.

Los resultados del cuarto experimento respaldan el efecto positivo de la hormona hCG en el crecimiento folicular, al indicar un aumento en el porcentaje de cerdas prepúberes con niveles de P4 ≥ 30 ng/mL cuando la PG600 fue suplementada con hCG 24 o 48 h después. Este aumento fue significativo solamente en las cerdas menores de 160 días de edad, lo que sugiere que a medida que las cerdas prepúberes maduran, la producción endógena de LH incrementa, de modo que la suplementación de la PG600 con hCG se vuelve innecesaria. Asi mismo, este dato también soporta los resultados de los experimentos 1 y 2, donde la respuesta ovulatoria inducida por la hormona eCG (sin actividad LH) aumentó con la edad y el peso de las hembras prepúberes. El incremento en la respuesta ovulatoria tras la suplementación de la PG600 con hCG también respalda previos estudios (Bolamba et al., 1991; Driancourt et al., 1992), donde se observó una mejora del celo y la ovulación cuando se aumentó la dosis de hCG administrada a cerdas prepúberes. El mayor aumento en los niveles de progesterona se observó cuando la hCG se administró a las 0 y las 24 h, sugieriendo que la dependencia de

los folículos en la LH podría disminuir tras el proceso de selección inicial, debido una mayor cantidad de IGF-1 que podría actuar sinérgicamente con la LH para promover las últimas fases del desarrollo folicular (Lucy et al., 2001).

Finalmente, los resultados del quinto experimento indican que el aumento en el porcentaje de cerdas ovuladas en respuesta a la suplementación de PG600 con hCG, asi como los niveles elevados de progesterona, son debidos al desarrollo de un mayor número de cuerpos lúteos, que está directamente relacionado con la dosis de hCG administrada. El aumento en el número de cuerpos lúteos podría estar asociado a una mayor producción endógena de andrógenos. En este sentido, la administración de testosterona en cerdas prepúberes aumentó el número de folículos preovulatorios y cuerpos lúteos, (Cárdenas and Pope, 1994; 1997), mientras que la superovulación del hámster mediante la administración de hCG estuvo asociada a un aumento en la síntesis de andrógenos a nivel folicular (Greenwald, 1993).

Además de un mayor número de cuerpos lúteos, la dosis más alta de hCG (200 IU) también indujo múltiples quistes foliculares en la mayoría de las cerdas, mientras que a menor dosis (100 IU) hubo una disminución tanto en la incidencia de cerdas con quistes, como en el número quistes por animal. Una relación similar entre los niveles de gonadotropinas y el desarrollo de quistes ováricos fue observada por Breen et al. (2005), que demostró un aumento en el número de quistes en cerdas nulíparas tratadas con 1.200 IU de PG600, en comparación con la administración de 900 o 600 IU. Es probable que el efecto negativo del exceso de hCG suceda a nivel ovárico, puesto que la mayoría de las cerdas tratadas con 200 IU de hCG presentaron cuerpos lúteos y quistes foliculares al mismo tiempo,

indicativo de que la señal ovulatoria fue recibida pero no actuó en todos los folículos. Teniendo en cuenta que el peso medio de las cerdas en este estudio fue de 106 kg, es posible que algunos de los animales fueses ya bastante maduros a nivel fisiológico para responder a la PG600 sola, de modo que la suplementación con hCG pudiera haber causado un exceso en la actividad LH conduciendo al desarrollo de los quistes ováricos. Un efecto perjudicial de la suplementación con hCG asociado a la edad de las cerdas fue también observado en un estudio anterior, cuando la inyección de 200 IU de hCG 24 h después PG600 disminuyó la fertilidad en cerdas de número de parto ≥ 3, pero no en los animales de primer o segundo parto (Anexo I).

Es posible que el exceso de hCG disminuyese la expresión de los receptores de LH en algunos folículos, tal como se ha demostrado en las células de la granulosa en la rata (LaPolt et al., 1990), lo que podría inhibir su respuesta al pico ovulatorio de LH induciendo el desarrollo de quistes ováricos. Alternativamente, es posible que un exceso de hCG, en presencia de actividad FSH residual de la PG600, indujese la selección de folículos accesorios en algunas animales, que no alcanzarían un desarrollo suficiente antes del pico de LH. En este sentido, la ecografía transrectal previa al tratamiento gonadotrópico indicó la presencia de folículos de 3 mm en algunos de los animales, que podrían responder directamente a la actividad de LH de la PG600, como se muestra por el 63 % de respuesta ovulatoria en el grupo que no recibió hCG suplementaria. La administración de 200 UI de hCG en estos animales puede haber seleccionado folículos adicionales no solo en el momento de la inyección, sino también en los días consecutivos, que con una velocidad de crecimiento de tan solo 1 mm al día (Morbeck et al., 1992), no llegarían a tiempo para ovular en el pico de LH, convirtiéndose en quistes foliculares. Aunque especulativo, la administración de 100 IU de hCG podría haber seleccionado folículos adicionales sólo en el momento de la

inyección, que serían menos propensos a desarrollar quistes en los animales que ya respondieron a la PG600, mientras que al mismo tiempo mejoraría el crecimiento folicular en el 37 % restante de las hembras.

Es posible que la administración suplementaria de dosis más bajas de hCG (20-50 IU), inyectada a las 24 horas después de PG600, sea capaz de seleccionar folículos en la mayor parte de las cerdas jóvenes prepúberes sin inducir la formación de quistes. Alternativamente, es posible que la administración de eCG sola pueda ser capaz de sincronizar el crecimiento de la mayoría de los folículos hasta los 4 mm de diámetro, cuando se volverían receptivos a la actividad LH de la hCG. De acuerdo con esta hipótesis, la administración de 400 IU de eCG, seguida de 200 IU de hCG 24 h después, indujo la ovulación en el 100% de los animales (experimento 3), mientras que muy pocas cerdas de 150 d mostraron niveles de progesterona por encima de 30ng/mL, lo que sugiere una respuesta ovulatoria normal.

Teniendo en cuenta todo lo anterior, podemos concluir que la actividad LH y FSH contenida en la PG600 se ha optimizado para inducir la ovulación en las cerdas jóvenes sin causar el desarrollo de quistes foliculares, aunque el 25-35% de los animales no responde al tratamiento. La suplementación con hCG consecutiva a la PG600 aumentó tanto el porcentaje de cerdas ovuladas como el número de cuerpos lúteos por ovario, pero su uso se asoció con el desarrollo de quistes ováricos de forma dependiente con la dosis de hCG. El mecanismo fisiológico y la implicación en la fertilidad de la administración adicional de hCG en cerdas necesita seguir siendo investigado.

8.3 Conclusiones

1. La falta de respuesta ovulatoria en el 30% de los animales tratados con PG600 no está asociada con una prematura ovulación o luteinización de los folículos inducida por la hormona hCG.

2. Las tasas de celo y ovulación las cerdas prepúberes inyectadas con gonadotropinas aumentas con la edad y el peso de los animales.

3. Las hormonas eCG y hCG administradas en solitario no son eficaces para estimular la ovulación en cerdas prepúberes, debido a su incapacidad para estimular el crecimiento de los folículos en alguna etapa del desarrollo.

4. La administración consecutiva de FSH y hCG produce una respuesta ovulatoria similar a la administración de PG600.

5. La suplementación de PG600 con hCG a las 24 h mejora la respuesta ovulatoria en cerdas prepúberes, y aumenta la concentración media posovulatoria de progesterona en sangre debido a la formación de un mayor número de cuerpos lúteos. La suplementación con hCG también produce un aumento en el número de quistes foliculares, que está directamente relacionado con la dosis administrada.

CAPÍTULO 10: Bibliografía

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ANEXOS

ANEXO 1: Effect of gonadotrophin treatment on estrus, ovulation and litter size in weaned sows

Published in *Journal Animal Science,* 2010. 88:2356-2360

Effect of gonadotropin treatment on estrus, ovulation, and litter size in weaned and anestrous sows[1]

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ABSTRACT: In the first of 2 experiments, we evaluated the effects on anestrous sows of pretreatment with FSH to stimulate the growth of small follicles, followed by eCG to stimulate the growth of medium follicles, estrus, and ovulation. In Exp. 2, we examined the effect of sows receiving 400 IU of eCG plus 200 IU of hCG (PG 600, Intervet/Schering Plough Animal Health, Boxmeer, the Netherlands) at weaning and then different doses and timing of supplemental hCG. In Exp. 1, a total of 87 multiparous Hypor sows deemed anestrus 7 d after weaning were assigned to intramuscular (i.m.) injection of 1) PG 600, 2) eCG (600 IU), 3) pretreatment with 87.5 IU of FSH on d 7 and 8 plus eCG on d 9, or were 4) noninjected controls. Sows had daily boar contact for 15 d after weaning for estrus detection. Blood samples were obtained on d 9 and 19 and assayed for progesterone to determine ovulation status. The weaning-to-estrus interval, number of sows in estrus and ovulating, farrowing rate, and litter size were not different ($P > 0.1$) in treated groups compared with controls. In Exp. 2, a total of 247 Hypor sows were assigned at weaning by parity (1 and 2 or ≥3) to receive 1) an i.m. injection of PG 600, 2) PG 600 supplemented with 100 IU of hCG injected either concurrently or after 24 h, 3) 200 IU of hCG after 24 h, or 4) no injection (controls). Sows were exposed to boars daily for 7 d. After treatment of parity 1 and 2 sows, all gonadotropin-treated groups had an increased ($P < 0.05$) number of sows in estrus compared with the control group; weaning-to-estrus interval, farrowing rates, and litter size were unaffected ($P > 0.1$). After treatment of parity ≥3 sows, there was no treatment effect on the estrous response and weaning-to-estrus interval; compared with control and PG 600-treated sows, farrowing rate was decreased ($P < 0.05$) for sows receiving 200 IU of hCG after 24 h. There was no effect ($P > 0.1$) of treatment on litter size. We conclude that gonadotropins can be used to increase estrus response in weaned sows, but that hCG treatment subsequent to PG 600 may be detrimental to sow fertility in parity ≥3 sows.

Key words: anestrus, farrowing, human chorionic gondaotropin, PG 600, sow

J. Anim. Sci. 2010. 88:2356–2360
doi:10.2527/jas.2009-2792

INTRODUCTION

In weaned sows, estrus can be induced by injecting 600 to 1,000 IU of eCG (Brussow et al., 2009) or a combination of 400 IU of eCG and 200 IU of hCG (PG 600, Intervet/Schering Plough Animal Health, Boxmeer, the Netherlands) on the day of weaning (Kirkwood et al., 1998). Estrus responses to treatment can be variable, and breeding to a hormone-induced estrus can result in decreased farrowing rates, smaller litters, or both (Estienne and Hartsock, 1998; Kirkwood, 1999). We have demonstrated in prepubertal gilts that hCG treatment subsequent to PG 600 increased early luteal blood progesterone concentrations (Manjarin et al., 2010), which may be a consequence of an increased number of corpora lutea or an increased progesterone output by individual corpora lutea. If numbers of ova released or embryo survival are increased because of enhanced luteal function, larger litter sizes may ensue. Therefore, we

[1]We gratefully acknowledge Intervet/Schering Plough Animal Health (Boxmeer, the Netherlands) for providing the PG 600 and Chorulon. We thank Union Veterinarios Españoles Sociedad Anonima (Malaga, Spain) for allowing access to the gilts, Evaristo Aguado (Villaquejida, Spain) for his expert assistance throughout the study, and Laboratorio de Tecnicas Instrumentals, University of Leon (Leon, Spain) for providing the laboratory facilities.

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Received December 28, 2009.
Accepted March 30, 2010.

hypothesized that the fertility of sows receiving PG 600 at weaning would be improved by injection of supplemental LH-like activity.

A more economical approach to reduce weaning-to-estrus intervals is by treating only those sows failing to exhibit a prompt return to estrus after weaning (Bracken et al., 2006), although treatment of anestrous sows often results in poorer fertility, possibly because of inadequate follicular development in a proportion of the anestrous sow population (Lucy et al., 2001; Bracken et al., 2003). Injection of FSH into gilts promoted growth in the small follicle populations (Guthrie et al., 1990; Bolamba et al., 1996; Knox, 2005). Therefore, we hypothesized that injection of FSH into anestrous sows would enhance the growth of small follicles, potentially generating a larger pool of medium follicles that would respond to an eCG injection. The objectives of these studies were first, to determine the effect of pretreatment with FSH on the reproductive responses of gonadotropin-treated anestrous sows, and second, to determine the effect of dose and timing of administration of supplemental hCG on reproductive responses of gonadotropin-treated weaned sows.

MATERIALS AND METHODS

Animals were cared for humanely in accordance with the University of Leon institutional animal care guidelines.

Exp. 1: Effect of Prior FSH Treatment on Response of Anestrous Sows to eCG

This study was performed on a 1,000-sow farrowing-to-weaning facility near Leon, Spain, during July, August, and September of 2007. At weaning, mixed-parity Hypor (Large White and Landrace breeding) sows were housed in individual gestation stalls and fed 3 kg/d of a gestation diet formulated to provide approximately 3,265 kcal of ME/kg and 0.55% total lysine. All sows received 5 min of daily fence-line boar contact from 2 d after weaning to facilitate estrus detection. At 7 d after weaning, 87 sows that had failed to exhibit estrus were allocated to 4 treatment groups balanced for parity and received 1) an intramuscular (**i.m.**) injection of 400 IU of eCG plus 200 IU of hCG (5 mL of PG 600, Intervet/Schering Plough Animal Health) on d 9 (n = 23); 2) 600 IU of eCG (3.0 mL of Folligon, Intervet/Schering Plough Animal Health) on d 9 (n = 20); 3) injection of 87.5 IU of FSH (Folltropin, Bioniche Animal Health, Belleville, Ontario, Canada) mixed with polyvinyl-pyrrolidinone C-30 (5 mL of **PVP**, Plasdone C-30, ISP Technologies Inc., Wayne, NJ; average molecular weight = 58,000) on d 7 and d 8, followed by 600 IU of eCG on d 9 (n = 24); or 4) noninjected controls (n = 20). Sows were not restrained for injection. The dose of eCG was chosen on the basis of known efficacy in weaned sows (Cassar et al., 2005). The objective of administering FSH in PVP solution was to prolong the period of biological activity of the hormone (Jackson et al., 2006). The PVP solution (30%) was prepared by dissolving 15 mg of PVP in 40 mL of distilled and deionized water; this was stored at 4°C until use.

Boar exposure for estrus detection continued for an additional 7 d, and estrous sows were artificially inseminated with 3×10^9 sperm from Centrotec (Campo de Villavidel, Leon, Spain) at detection of estrus and at 24-h intervals if still exhibiting estrus. All sows received a minimum of 2 matings. Bred sows were allowed to go to term to determine farrowing rates and subsequent litter sizes. To determine the ovulation responses to hormone treatment, blood samples (8 mL) were collected, and the serum was harvested within 4 h by centrifugation at 2,000 × *g* for 4°C for 15 min. Samples were obtained from all sows via jugular venipuncture at 9 and 19 d after weaning and analyzed for progesterone concentrations in a single assay using a commercial ELISA kit (Immulite, Siemens Medical Solutions Diagnostics, Tarrytown, NY). Assay sensitivity and intraassay CV were 0.2 ng/mL and 8.1%, respectively. An increase in progesterone concentrations from <1 ng/mL on d 9 to a minimum of 5 ng/mL on d 19 was considered to indicate an ovulation response to treatment (Althouse and Hixon, 1999).

Exp. 2: Effect of Supplemental hCG on Response of Weaned Sows to PG 600

This study was performed at the same facility as above during July, August, and September of 2008. A total of 247 mixed-parity Hypor sows were weaned into individual gestation stalls and fed 3 kg/d of a gestation diet formulated to provide approximately 3,265 kcal of ME/kg and 0.55% total lysine. Sows were assigned by parity (1 and 2 vs. ≥3) to receive an i.m. injection at weaning of 1) 400 IU of eCG plus 200 IU of hCG (5 mL of PG 600, Intervet/Schering Plough Animal Health; n = 15 for parities 1 to 2, and n = 40 for parity ≥3, respectively), 2) PG 600 with 100 IU of hCG (0.33 mL of Chorulon, Intervet/Schering Plough Animal Health) injected i.m., either concurrently (n = 14 and 34, respectively) or after 24 h (n = 18 and 32, respectively), or 3) 200 IU of hCG (0.66 mL) injected 24 h after PG 600 (n = 14 and 31, respectively). Sows were not restrained for injection. An additional 54 sows served as noninjected controls (n = 14 and 34, respectively). The hCG doses were chosen on the basis of observed efficacy in a gilt model (Manjarin et al., 2010). All sows received 5 min of daily fence-line boar contact for a period of 7 d after weaning to facilitate estrus detection. Estrous sows were artificially inseminated with 3×10^9 sperm (Centrotec) at detection of estrus and at 24-h intervals if still exhibiting estrus. All sows received a minimum of 2 matings. Sows were allowed to go to term to determine farrowing rates and subsequent litter sizes.

Table 1. Effect of injection of 600 IU of eCG[1] and 87.5 IU of FSH[2] on d 2, followed by 600 IU of eCG or PG 600[3] on the estrus and ovulation responses of anestrous sows at 7 d after weaning

Item	Control	eCG	FSH + eCG	PG 600
No. of sows	20	20	24	23
Parity[4]	3.9 ± 0.4	3.7 ± 0.4	3.7 ± 0.4	3.6 ± 0.4
Weaning-to-estrus interval,[4] d	4.6 ± 0.5	4.3 ± 0.6	4.1 ± 0.7	4.2 ± 0.5
Estrus,[5] %	3 (15.0)	9 (45.0)	13 (54.1)	10 (43.4)
Ovulation,[6] %	4 (20.0)	9 (45.0)	12 (50.0)	12 (52.1)
Farrowing,[7] %	3 (100)	7 (77.8)	11 (84.6)	10 (100.0)
Litter size[4]	9.0 ± 2.5	10.0 ± 3.7	9.5 ± 3.4	9.1 ± 2.8

[1]Folligon (Intervet/Schering Plough Animal Health, Boxmeer, the Netherlands).
[2]Folltropin (Bioniche Animal Health, Belleville, Ontario, Canada).
[3]Intervet/Schering Plough Animal Health.
[4]Means ± SE.
[5]Sows in estrus by 7 d after treatment (% values from total number of sows in parentheses).
[6]Ovulation indicated by serum progesterone >5 ng/mL (% values from total number of sows in parentheses).
[7]Data in parentheses are percentages.

Statistical Analyses

In Exp. 1, the effect of gonadotropin treatment on the response variables was analyzed using a generalized mixed model (PROC GLIMMIX, SAS Inst. Inc., Cary, NC). Both treatment and parity factors were included as fixed effects. Response variables, including frequency of estrus and ovulation and sows farrowing, were expressed as the number of cases assuming a binary distribution. The effect of treatment on weaning-to-estrus interval and litter size was analyzed using the mixed model procedure (PROC MIXED) of SAS, assuming a normal distribution and including the effect of parity as a fixed term. A value of $P < 0.05$ was set as the significant level in testing all ANOVA results. Mean differences for all treatments vs. the control were computed and reported.

In Exp. 2, the effect of gonadotropin treatment on the response variables was analyzed using a generalized mixed model, with treatment and parity as fixed effects. Response variables, including frequency of estrus and sows farrowing, were expressed as the number of cases, assuming a binary distribution. The effect of treatment on weaning-to-estrus interval and litter size was analyzed using the mixed model procedure including the effect of parity as a fixed term. A value of $P < 0.05$ was set as the significant level in testing ANOVA results. Mean differences for all treatments vs. the control were computed and reported.

RESULTS

Exp. 1

The effect of parity was not significant. All sows used in this study had nondetectable progesterone concentrations on d 9 after weaning, whereas all sows determined to have ovulated had circulating progesterone concentrations of >20 ng/mL on d 19. There was no effect of treatment on weaning-to-estrus interval, estrus, ovulation, farrowing rate, or litter size (Table 1).

Exp. 2

The percentage of parity 1 and 2 sows returning to estrus within 7 d after weaning was influenced by treatment, with all gonadotropin-treated groups increasing ($P < 0.05$) the estrus response compared with the control group (Table 2). There was no effect of treatment on weaning-to-estrus interval, farrowing rate, or subsequent litter size. Treatment of parity ≥3 sows had no effect on weaning-to-estrus interval, estrus response, or litter size (Table 2). However, compared with control sows and sows receiving PG 600, farrowing rate was decreased ($P < 0.05$) for sows injected with 200 IU of hCG 24 h after PG 600.

DISCUSSION

In Exp. 1, we hypothesized that sows pretreated with FSH would have greater estrus, ovulatory, and farrowing responses to eCG injection, presumably because of enhanced follicular development, but this was not evident. Although we did not measure ovarian follicle growth in this study, based on a previously published similar FSH-treatment protocol (Jackson et al., 2006), it is likely that even those sows that failed to ovulate had early antral follicle growth initiated by FSH treatment. In turn, this likely indicates that sows pretreated with FSH that failed to respond to eCG treatment may lack adequate endogenous LH-like activity to maintain follicle development to preovulatory size. Indeed, nutritionally driven (and presumably seasonally driven) delayed estrus was associated with a reduced basal concentration of LH (Kirkwood et al., 1987), and appropriate LH concentrations are required for follicle development beyond 4 mm (Driancourt et al., 1995, Liu et al., 2000). Interestingly, PG 600 contains 200

Table 2. Influence of parity on the effect of PG 600[1] and PG 600 followed by supplemental hCG[2] on estrus and farrowing rates, and subsequent litter size in weaned sows[3]

Item	Control	PG 600	PG 600 + 100hCG-0[4]	PG 600 + 100hCG-24[5]	PG 600 + 200hCG-24[6]
Parities 1 and 2					
No. of sows	14	15	14	18	14
Weaning-to-estrus interval,[7] d	4.8 ± 0.4	4.1 ± 0.7	4.3 ± 0.6	4.3 ± 0.3	4.3 ± 0.6
Estrus, %	9 (64.3)[a]	15 (100)[b]	14 (100)[b]	17 (94.4)[b]	14 (100)[b]
Farrowing, %	5 (55.5)	12 (80.0)	8 (57.1)	13 (76.5)	11 (78.6)
Litter size[6]	7.8 ± 2.4	8.4 ± 2.4	9.4 ± 2.7	10.1 ± 4.0	9.4 ± 4.3
Parity ≥3					
No. of sows	34	41	34	32	31
Weaning-to-estrus interval,[7] d	4.6 ± 0.6	4.2 ± 0.6	4.2 ± 0.7	4.0 ± 0.5	4.1 ± 0.3
Estrus, %	28 (82.3)	39 (95.1)	30 (88.2)	32 (100)	30 (96.8)
Farrowing, %	23 (82.1)[a]	34 (87.2)[a]	23 (76.7)[ab]	24 (75.0)[ab]	17 (56.7)[b]
Litter size[7]	10.0 ± 1.8	10.4 ± 1.8	10.0 ± 2.1	10.2 ± 1.8	9.8 ± 1.5

[a,b]Means within row followed by different letters are different ($P < 0.05$).
[1]Intervet/Schering Plough Animal Health (Boxmeer, the Netherlands).
[2]Chorulon (Intervet/Schering Plough Animal Health).
[3]Data in parentheses are percentages.
[4]PG 600 injected concurrently with 100 IU of hCG.
[5]PG 600 followed after 24 h with 100 IU of hCG.
[6]PG 600 followed after 24 h with 200 IU of hCG.
[7]Means ± SE.

IU of hCG, and we observed no difference in estrus or ovulation responses between eCG- and PG 600-treated anestrous sows. However, previous work has demonstrated that even when treating sows with PG 600, some endogenous secretion of LH is required to sustain follicle growth, and the preovulatory LH surge is entirely endogenous (Kraeling et al., 1990; Garcia et al., 2004). Therefore, the less than 50% estrus response to gonadotropic stimulation of anestrous sows in the present study, compared with 70% or greater in sows treated with gonadotropins at weaning (Britt et al., 1986; Bates et al., 1991; Kirkwood et al., 1995, 1998), may be due to anestrous sows needing a more prolonged period of LH-like activity than that provided by PG 600 to stimulate and maintain ovarian follicular growth.

Bracken et al. (2006) reported a 93% estrus response of anestrus sows to PG 600 injection 7 d after weaning. The reason for the difference in response rates compared with the present study (43.4%) is not known. However, the response of anestrous sows to hormonal stimulation will depend on the degree of follicular development at the time of treatment. The mean diameter of ovarian follicles in the study by Bracken et al. (2006) was approximately 4 mm, a follicle size associated with a transition to LH control (Driancourt et al., 1995), and thus greater responsiveness to the hCG component of PG 600. In contrast, heat-stressed sows had follicles of only <3 mm in diameter (Lucy et al., 2001; Bracken et al., 2003), and historically, sows on our study farm have exhibited marked seasonal infertility. However, we did not measure follicle size in the present study, so a comparison between the sow populations was not possible.

The results from Exp. 2 indicate that supplementing weaned sows with different amounts of LH-like activity at different stages of a follicular phase induced by PG 600 did not improve estrus response, pregnancy rate, farrowing rate, or litter size compared with treatment with PG 600 alone. This result was not completely unexpected because in our previous work (Manjarin et al., 2010), we found that the effect of supplemental LH-like activity on progesterone concentrations in prepubertal gilts was age dependent, being less evident as the population aged and with the endogenous LH support likely becoming adequate to support follicular development. In the present study, we found that injecting younger sows with PG 600 increased estrus response rates compared with injecting control sows, but there was no effect of supplemental hCG. In contrast, PG 600 did not affect the estrus responses of older sows, but farrowing rate was reduced in the sows receiving 200 IU of supplemental hCG. It is possible that more sows injected with 200 IU of hCG 24 h after PG 600 expressed estrous behavior without ovulation, which would manifest as a reduced farrowing rate. Further, it is possible that an excess of hCG in older sows down-regulated follicular LH receptors, as was shown to occur in rat granulosa cells (LaPolt et al., 1990), which could have the effect of inhibiting ovulation. It is also possible that the supplemental hCG interfered with the maintenance of pregnancy because of cyst formation. Indeed, we recently noted a high incidence of cysts in PG 600-treated prepubertal gilts receiving 200 IU of supplemental hCG 24 h later (J. C. Garcia, unpublished data), and it was shown previously that administration of PG 600 at greater doses than recommended induced cysts (Breen et al., 2006). Follicular cysts in sows act as a primary source of progesterone (Close and Liptrap, 1975), and inappropriately timed increases of progesterone could result in a uterine milieu not conducive to embryo development.

From the above discussion, we suggest that responsiveness of anestrus sows to exogenous gonadotropins

will depend on the combined effects of initial follicle size and ongoing luteotrophic support. Anestrus sows are likely to have reduced endogenous gonadotropin concentrations, which could limit their ability to support ongoing follicular development as well as populations of smaller size follicles. We speculate that under these conditions, the injection of supplemental hCG is more likely to enhance the response to PG 600.

We conclude from the present data that supplemental LH-like activity does not improve the fertility rate when added to the PG 600 treatment 24 h after weaning and is therefore not indicated as a common protocol for the induction of estrus and ovulation in weaned sows. We suggest that given the dynamic changes in follicular control mechanisms around the 4-mm follicle stage, it is possible that currently available gonadotropin preparations or their modes of administration may be inappropriate for many seasonally anestrus sows. An improved understanding of the effect of LH on follicular growth may facilitate the development of improved protocols for hormonal induction of estrus in this population of sows.

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ANEXO 2: Curriculum Vitae

Publicaciones Cientificas-Peer-reviewed publications

Manjarin, R., Cassar, G., Friendship, R.M., Garcia J.C., Dominguez, J.C., Kirkwood, R.N. 2013. Effect of additional hCG on follicular growth and ovulation in PG600-treated gilts. Submitted, J. Anim. Sci.

Faramarzi, B., Halland, S., Dobson, **Manjarin, R.,** H., Kaneps, A., and McMicking, H.F. Incidence of fractures of the palmar processes of distal phalanx in Thoroughbred, Quarter Horse, and Arabian foals in Southern California. Submitted JAVMA

Hidalgo, D.M., Dominguez, J.C., **Manjarin, R.**, Cassar, G., Friendship, R.M., Kirkwood, R. N. 2013. Examination of gilt characteristics associated with subsequent fertility. Submitted. J. Anim. Sci.

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VanKlompenberg, M.K, **Manjarin, R.**, McMicking, H.F., Hovey, R.C. 2013. Late gestational hyperprolactinemia enhances mammary gland vasculature. Submission date: December 2013.

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Premios-Honors and awards

2012. American Society of Animal Science Wilson Pond International Travel Award

2011. American Society of Animal Science Midwest Section Young Scholar