UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA VETERINÁRIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

INSEMINAÇÃO ARTIFICIAL EM TEMPO FIXO EM LEITOAS E PORCAS DESMAMADAS COM O USO DE HORMONIO LUTEINIZANTE SUÍNO ATRAVÉS DE DIFERENTES VIAS DE APLICAÇÃO

RAFAEL DA ROSA ULGUIM

PORTO ALEGRE

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Orientador: Dr. Ivo Wentz

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Resumo

A redução do número de doses inseminantes por fêmea coberta utilizando protocolos de uma única inseminação em tempo fixo (IATF) permitem reduzir o número de células espermáticas por fêmea coberta e otimizar os programas de inseminação artificial (IA). Considerando a grande variabilidade no intervalo entre o início do estro e a ovulação, os protocolos de IATF exigem hormonios para a sincronização da ovulação. Assim, o objetivo deste estudo foi avaliar a utilização de diferentes dosagens de hormônio luteinizante suíno (pLH) aplicado no início do estro em leitoas e porcas, através de diferentes vias de aplicação, para sincronização da ovulação e definição de um protocolo de IATF. O primeiro estudo avaliou o efeito de diferentes dosagens de pLH aplicado em leitoas no início do estro por via intramuscular (i.m.), sobre o intervalo início estro e a ovulação. Desta forma foram realizados três tratamentos: controle - sem aplicação de pLH no início do estro; pLH2,5 - uso de 2,5 mg de pLH no início do estro via i.m.; pLH5 - uso de 5 mg de pLH no início do estro via i.m. Não foram observadas diferenças no intervalo início do estro e a ovulação (IOEO) entre os diferentes tratamentos (P>0,05). De forma semelhante a distribuição de frequência do IOEO não diferiu entre os tratamentos (P>0,05). Em um segundo estudo, foi avaliado uma rota alternativa de aplicação de pLH e a performance reprodutiva de leitoas submetidas a uma única IATF. Assim, os seguintes tratamentos foram realizados: Controle - sem aplicação de hormônio no inicio do estro e realização de protocolos de múltiplas IAs; VS2.5FTAI - uso de 2,5 mg de pLH aplicado no início do estro via submucosa vulvar (v.s.) e realização de uma única IATF 16 h após; IM5FTAI - uso de 5 mg de pLH aplicado no inicio do estro via i.m. e realização de uma única IATF 16 h após. Em média foram observadas diferenças no IOEO entre os tratamentos (P<0,05) e maior frequência de leitoas ovuladas até 24 h após o inicio do estro no grupo VS2.5FTAI em relação ao grupo controle (P<0,05). A taxa de parto ajustada (AFR) não diferiu entre tratamentos (P>0,05), porém o total de leitões nascidos (TPB) foi menor no grupo VS2.5FTAI em relação ao grupo controle (P<0,05). Com objetivo de ajustar o protocolo de IATF em leitoas para uma melhor aplicabilidade prática na rotina das granjas e avaliar o uso do pLH via v.s. em porcas desmamadas, o terceiro estudo foi conduzido através de dois experimentos. Nas leitoas foram realizados dois tratamentos: controle-G - sem uso de pLH no início do estro e realização de múltiplas inseminações ao longo do estro; FTAI-G - aplicação de 2,5 mg de pLH via v.s. no início do estro e realização de

uma única IATF 12 h após. O IOEO foi menor nas leitoas do grupo FTAI-G comparado ao controle-G (P<0,05), no entanto a distribuição de frequência do IOEO não foi diferente entre os tratamentos (P>0,05). A AFR foi menor para o grupo FTAI-G quando comprado ao controle-G (P<0,05). Diferenças no TPB não foram observadas entre tratamentos (P>0,05). Nas porcas desmamadas, foram realizados três tratamentos: Controle-S - sem aplicação de pLH no início do estro e realização de múltiplas inseminações; FTAI-NH - sem aplicação de pLH no início do estro e realização de uma única inseminação 24 h após; FTAI-pLH - uso de 2,5 mg de pLH no início do estro via v.s. e realização de uma única inseminação 24 h após. Os resultados deste estudo não asseguraram diferença quanto a AFR e TPB entre os distintos tratamentos (P>0,05).

Palavras chave: sincronização da ovulação, indução da ovulação, momento da ovulação, reprodução em suínos, inseminação em suínos.

Abstract

The reduction in the number of semen doses used per sow served through of a single fixed-time insemination (FTAI) allows sperm cell reduction per sow served, optimising the artificial insemination (AI) programs. Considering the large variability in the interval between oestrus onset and ovulation the ovulation time, the FTAI protocols require hormones to synchronise ovulation. Thus, the aim of this study was to evaluate the use of different dosages of porcine luteinising hormone (pLH) given at oestrous onset in gilts and sows through different routes of application to synchronise the ovulation and to define a protocol of FTAI. The first study evaluated the effect of different dosages of pLH applied at oestrous onset by intramuscular route (i.m.) in gilts on interval between oestrus onset to ovulation. In this way three treatments were performed: control - without pLH application at oestrous onset; pLH2.5 - use of 2.5 mg of pLH given at oestrous onset by i.m. route; pLH5 - use of 5 mg of pLH given at oestrus onset by i.m. route. Differences in the interval onset of oestrus to ovulation (IOEO) among treatments ($P>0.05$) were not observed. Similarly the frequency distribution of IOEO did not differ among treatments ($P>0.05$). In a second study, was evaluated an alternative route of pLH application and the reproductive performance of gilts submitted to a single FTAI. Thus, the following treatments were performed: control – without pLH application at oestrous onset and use of multiple AI; VS2.5FTAI – use of 2.5 mg of pLH injected at oestrus onset by vulvar submucosal route (v.s) and use of a single FTAI 16 h later; IM5FTAI – use of 5 mg of pLH injected at oestrous onset by i.m. and use of a single FTAI 16 h later. On average differences in the IOEO among treatments ($P<0.05$) were observed and more VS2.5FTAI gilts ovulated up to 24 h after oestrous onset in relation to control ($P<0.05$). Adjusted farrowing rate (AFR) did not differ among treatments ($P>0.05$), however the total piglets born (TPB) was lower in the group VS2.5FTAI compared to control ($P<0.05$). In order to adjust the FTAI protocol in gilts for a practical use in the routine of the farm and to evaluate the use of pLH by v.s. route in weaned sows, the third study was conducted through two experiments. In gilts two treatments were performed: control-G - without pLH injection at oestrous onset and use of multiple AI during the oestrous; FTAI-G – 2.5 mg pLH applied by v.s. route at oestrous onset and use of a single FTAI 12 h later. The IOEO was shorter in the FTAI-G gilts compared to control-G ($P<0.05$), but the frequency distribution of IOEO did not differ between treatment ($P>0.05$). The AFR was lower to

FTAI-G group compared to control-G ($P<0.05$). Differences on TPB between treatments were not observed ($P>0.05$). In the weaned sows three treatments were performed: control-S - without pLH application at oestrous onset and use of multiple inseminations; FTAI-NH - no hormone application and a single FTAI 24h after the onset of oestrus detection; FTAI-pLH - use of 2.5 mg pLH at oestrous onset by v.s. route and use of a single FTAI 24 h later. The results of this study did not insure difference on the AFR and TPB among treatments ($P>0.05$).

Keywords: synchronisation of ovulation, ovulation induction, ovulation time, swine reproduction, swine insemination.

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1. INTRODUÇÃO

Atualmente a inseminação artificial (IA) é uma tecnologia utilizada pela maioria dos sistemas de produção comercial de suínos. Ao longo dos anos, as pesquisas buscaram otimizar o uso desta biotécnica reprodutiva em suínos com objetivo de melhorar a qualidade das doses inseminantes (DI), reduzir custos, bem como otimizar o uso dos machos em centrais de IA.

A deposição cervical da dose de sêmen é o método mais utilizado na IA em suínos. A DI neste método possui de 3 a 4 bilhões de espermatozoides em um volume de 80 a 100 ml, podendo ser armazenada por três a cinco dias entre 15 a 18ºC. Diferentes protocolos de IA podem ser utilizados baseados no número de identificações diárias de estro e na categoria da fêmea, sendo que na grande maioria dos protocolos cada fêmea recebe até três doses de sêmen, totalizando 9 a 12 bilhões de espermatozoides/fêmea coberta (Bortolozzo et al., 2005a).

A busca pela redução dos custos e a otimização do uso dos machos impulsionou trabalhos no sentido de diminuir o número de células espermáticas utilizadas por fêmea inseminada. Desta forma, o uso de um método de IA que proporcionasse a deposição pós-cervical da DI foi desenvolvido (inseminação intra-uterina - IAU), possibilitando a redução do número de células espermáticas e volume das DI.

Nos últimos anos a utilização da IAU nos sitemas produtivos aumentou de forma expressiva. Durante alguns anos a técnica foi indicada somente para fêmeas com 2 ou mais partos, em função do reduzido tamanho do trato reprodutivo que dificulta a inserção do cateter intrauterino em fêmeas mais jovens (Araújo et al., 2009). No entanto, recentemente Sbardella et al. (2013) demonstraram a possibilidade e o sucesso de inserção do cateter intrauterino em primíparas sem comprometimento do desempenho reprodutivo. Desta forma, até o momento o uso da IAU permanece limitado em leitoas em função da dificuldade de inserção do cateter intrauterino.

A redução no número de DI ao longo do estro é outra possibilidade promissora para reduzir o número de células espermáticas por fêmea inseminada. A longa duração do estro em fêmeas suínas associada à grande variabilidade do momento da ovulação exige que as inseminações sejam realizadas em intervalos que proporcionem a manutenção de um número de células espermáticas viáveis no trato reprodutivo no momento da ovulação, elevando o número de DI por fêmea inseminada. Desta forma, algumas IAs são realizadas antes e após o momento considerado ideal (próximo a

ovulação), o que configura desperdício e custo com doses que não são efetivas no processo de fertilização. Segundo Castagna et al. (2003), na rotina de granjas comerciais de suínos mais de 70% das fêmeas recebem pelo menos uma IA após a ovulação, independentemente da frequência de realização desta. Em leitoas a variabilidade no momento da ovulação é maior em relação às demais categorias de fêmeas (Martini, 1998; Uemoto, 1999), o que exige um manejo de detecção de estro e inseminações mais frequentes para esta categoria. Assim, a manipulação do momento da ovulação por meio do uso de hormônios indutores da ovulação permite a redução da variabilidade do momento deste evento e a realização de uma única inseminação artificial em tempo fixo (IATF), no período mais próximo da ovulação (Cassar et al., 2005).

Em função das limitações existentes para o uso da IAU em leitoas, as possibilidades de redução no número de células espermáticas/fêmea coberta ficam restritas para esta categoria, sendo a redução no número de DI a única possibilidade nestas fêmeas. Segundo Zack et al. (2011) e Fontana et al. (2014) os resultados produtivos de uma única IATF em porcas, com o uso de 5 mg de hormônio luteinizante suíno (pLH) aplicado via intramuscular, não são alterados em comparação com protocolos tradicionais de IA que usam de 2 a 3 doses de sêmen por fêmea. A recomendação técnica do uso de pLH (Lutropin-V®[1]) em suínos, orienta a aplicação intramuscular de 5 mg do produto. Assim, embora os resultados produtivos não demonstrem diferença significativa na produtividade com o uso de uma única dose de sêmen, o custo do hormônio pode se tornar uma limitação ao uso da tecnologia. A busca pela redução da dose de pLH através de uma nova via de aplicação, pode contribuir para reduzir o custo do protocolo, mão de obra de aplicação e viabilizar a tecnologia de IATF em leitoas e porcas.

A possibilidade de redução do número de DI e do número de células espermáticas por fêmea coberta é uma alternativa que permite uma maior eficiência no programa de IA em suínos. Considerando que as leitoas constituem um percentual representativo de fêmeas no grupo de cobertura semanal, a adoção do uso de uma única dose em tempo fixo permitiria aumentar o número de fêmeas atendidas por macho. Isto permite a diminuição do número de machos na central e consequentemente redução de custos de mão-de-obra e manutenção destes animais no plantel, o que possibilita o aumento da difusão de machos geneticamente superiores e redução de custos no programa de IA em suínos.

[1] Bioniche Animal Health, Belleville, Ontário, Canada

2. CAPÍTULO I - REVISÃO BIBLIOGRÁFICA

1. Situação atual e perspectivas no uso da inseminação artificial em suínos

Programas de inseminação artificial (IA) são consolidados na produção comercial de suínos, observando-se ao longo da década de 90 um aumento progressivo anual na utilização desta biotécnica, sendo que em 2000 foi estimado que 51% das granjas tecnificadas utilizavam esta tecnologia (Wentz, et al., 2000). Hoje, possivelmente este percentual aumentou significativamente, muito embora dados estatísticos oficiais que espelhem esta possibilidade, são escassos. Estima-se que mais de 80% do rebanho industrial de suínos utiliza a técnica de inseminação (Wentz e Bortolozzo, informação pessoal).

A implantação de programas de inseminação artificial nas granjas, permitiu a maximização do uso dos machos e um maior controle de qualidade dos ejaculados. A técnica de deposição cervical da dose inseminante (inseminação artificial tradicional - IAT), foi precursora na implantação da IA no sistema produtivo de suínos e ainda permanece a técnica mais comumente utilizada na rotina produtiva. Na IAT são utilizadas doses de sêmen com 80 a 100 mL de volume, com um total de três bilhões de células espermáticas, que são depositadas 2 a 3 vezes ao longo do estro (Bortolozzo et al., 2008). O número de doses inseminantes realizadas por fêmea e o número de células espermáticas utilizadas por dose na técnica de IAT eleva o número de células espermáticas/fêmea/ano o que representa maior custo e mão-de-obra nos programas de IA.

Em função do elevado número de doses e células espermáticas utilizadas por fêmea inseminada na IAT, estratégias que buscam reduzir o número de células espermáticas/fêmea/ano, associadas a redução do número de doses/fêmea inseminada, têm sido foco das pesquisas em IA em suínos (Bortolozzo et al., 2008). Neste sentido, métodos alternativos de execução da técnica de IA, bem como de protocolos de IA foram desenvolvidos. Nos últimos anos, a otimização de mão-de-obra nos programas de IA estão sendo considerados através da avaliação de técnicas que permitem reduzir as atividades de detecção de estro, tempo de realização da IA e número de doses inseminantes por fêmea coberta. Neste contexto, uso da IA intra-uterina (IAU), bem como a utilização de protocolos que permitem a realização de uma única inseminação em tempo fixo (IATF), são alternativas que permitem alcançar estes objetivos. A IAU é

uma técnica que atualmente está em implantação no sistema produtivo e a IATF em fase de avaliação científica e serão discutidas detalhadamente na sequencia desta revisão.

2. Alternativas para redução do número de células espermáticas na dose inseminante

A utilização da IAT exige um volume e número de células espermáticas elevado nas DI, devido ao fato de muitos espermatozoides serem perdidos durante o processamento e armazenamento das doses, assim como no momento da inseminação e durante o trânsito no trato reprodutivo (Vasquez et al., 2005). Para que o processo de fertilização ocorra de forma ideal é necessário que exista uma quantidade adequada de espermatozoides viáveis no reservatório espermático (oviduto) no momento da ovulação. Portanto, perdas que ocorrem durante o transporte espermático no trato reprodutivo feminino influenciam a colonização do reservatório espermático e por consequência o processo de fertilização (Vaquez et al., 2005). Isto pode ser observado no trabalho de Guthrie et al. (2002), que verificaram resultados de fertilidade normal quando baixo número de espermatozoides foram selecionados por citometria de fluxo e cirurgicamente inseminados no oviduto. Desta forma, o local de deposição da DI no trato reprodutivo feminino exerce efeito na perda de células espermáticas durante o transporte espermático.

As perdas de espermatozoides no útero são principalmente relacionadas ao refluxo e fagocitose, sendo que em torno de 70% do volume e 25% a 45% dos espermatozoides infundidos são perdidos por refluxo (Steverink et al., 1998). O refluxo depende basicamente do local de deposição da DI e do tamanho do útero em relação ao volume de sêmen infundido (Baker e Degen, 1972), sendo que fêmeas primíparas possuem mais refluxo que fêmeas mais velhas (Steverink et al., 1998). Com relação a fagocitose, estima-se uma redução no número de espermatozoides a quantidades menores que 1% da infusão inicial até 24 horas após a inseminação (Woelders & Matthijs, 2001). Devido a isto, melhores taxas reprodutivas com reduzido número de células espermáticas são esperadas se a IA é realizada o mais profundo possível (Vasquez et al., 2005). Em função destas considerações foram desenvolvidas tecnologias de IA pós-cervical, com auxílio de pipetas e catéteres que permitem a IA intra-uterina no corpo do útero (IAU) e intra-uterina profunda (IAUP) mais próximo da junção útero-tubárica.

2.1 Inseminação artificial intra-uterina (IAU)

Neste método de IA o objetivo é depositar as células espermáticas diretamente no lúmen uterino, aproximadamente 20 a 25 cm após a cérvix (Watson & Behan, 2002), possibilitando a redução do número de espermatozoides por DI (Martinez et al., 2001). Esta técnica é realizada através do uso de um cateter de menor calibre que desliza pelo interior de uma pipeta tradicional, possibilitando a passagem pela via cervical. Com isto, é possível o emprego de doses com um bilhão de espermatozoides em um volume total de 50-60mL, o que corresponde a 1/3 do total de células empregadas nas doses com IAT e uma redução de 25-30% no volume de diluente consumido pela central (Watson & Behan, 2002).

A IAU, quando comparada com IAT apresenta algumas vantagens, como redução do número de espermatozoides por DI, sendo que trabalhos que compararam os métodos de IA e a redução no número de espermatozoides demonstraram ser possível trabalhar com um número inferior a três bilhões de espermatozoides por DI (Tabela 1). Como consequência da redução de células espermáticas na DI, há um aumento no número de DI produzidas de um mesmo ejaculado, otimizando o uso de machos geneticamente superiores e incrementando o ganho genético do rebanho. Além disso, devido ao fato do sêmen ser depositado após a cérvix, observa-se uma redução ou ausência de refluxo de sêmen durante a IAU (Dallanora, 2004).

Tabela 1- Desempenho de fêmeas inseminadas pela técnica de inseminação artificial intra-uterina (adaptado Bennemanm, 2008)

Trat	Sptz($x10^9$)	Vol (mL)	TPr(%)	TP(%)	NT/ET	Autor
IAU	1,5	60	-	94,9	11,5	Dallanora et al., 2004
IAT	3,0	90	-	94,4	11,76	
IAU	1,0	80	-	86,9	12,1	Watson & Behan, 2002
IAT	3,0	80	-	92,5	12,3	
IAU	0,5	20	-	92,7	11,3	Bennemann et al., 2005
IAT	3,0	20	-	95,1	12,1	
IAU	0,5	20	85,5	-	14,3	Mezalira et al., 2003
	1,0	20	84,7	-	13,3	
IAU	1,0	60	82,1	-	15,9	Bennemann et al., 2004
	2,0	60	96,5	-	14,9	

IAU-inseminação intra-uterina; IAT – inseminação tradicional; Sptz – espermatozóides; Vol – volume; TPr – taxa de prenhez; TP – taxa de parição; NT – leitões nascidos totais; ET – embriões totais; Trat – tratamento.

No entanto, existem desvantagens que podem comprometer ou limitar o uso da IAU, dentre elas a dificuldade de introdução do cateter em algumas fêmeas. Watson & Behan (2002), usando pipeta tipo Melrose com cateter intrauterino, obtiveram sucesso na passagem pela cervix em 95% das fêmeas, sendo que resultado semelhante foi observado por Dallanora (2004), que obteve 97,4% de sucesso. Porém, uma das particularidades destes trabalhos é a utilização desta técnica somente em fêmeas com ordem de parto ≥ 2. Recentemente Sbardella et al. (2014) mostraram a possibilidade de introdução do cateter intrauterino e realização da IAU em 86% das fêmeas primíparas, sem comprometer o desempenho reprodutivo quando comparado ao método de IAT. Estes resultados asseguraram a aplicação prática da IAU em fêmeas primíparas. No entanto, informações científicas quanto a utilização da IAU em leitoas permanecem escassas na literatura.

No plantel produtivo, as leitoas representam aproximadamente 20% do grupo de cobertura semanal, sendo que alternativas para redução no número de células espermáticas por fêmea coberta são limitadas para esta categoria em função da impossibilidade do uso da IAU e da falta ou escassez de informações a cerca do uso de protocolos de inseminação que utilizam um menor número de DI por leitoa coberta.

2.2 Inseminação artificial intra-uterina profunda (IAUP)

O uso da IAUP tem como objetivo reduzir ainda mais o número de espermatozoides por DI, por meio da deposição do sêmen próximo a junção útero-tubárica, mais próximo ao local de fertilização. O principal obstáculo para realização desta técnica é a anatomia do trato reprodutivo, caracterizado pelas dobras cervicais e pelo comprimento e característica sinuosa dos cornos uterinos, que dificultam o desenvolvimento de um cateter não cirúrgico para inserção através dos cornos uterinos (Vasquez et al., 2005).

Uma técnica não-cirúrgica para avaliar a possibilidade de inserção intra-uterina profunda do cateter foi desenvolvida por Martinez et al. (2001) utilizando um endoscópio flexível modificado de 1,35m. Foi observado no estudo, que o endoscópio progrediu sem dificuldade ao longo da cérvix e corno uterino, sendo que a inserção foi possível em 90% das porcas. Posteriormente, o mesmo pesquisador demonstrou resultados produtivos da IAUP com uso do endoscópio e doses de 50, 100 e 200 x 10^6 espermatozoides, comparado à IAT com DI de três bilhões de células espermáticas, não sendo observada diferença entre os grupos. A técnica da IAUP, apesar de mostrar-se

efetiva, é inviável no uso a campo devido ao custo elevado e a fragilidade do material de que é feito o endoscópio (Martinez et al., 2001).

Após o uso da IA com endoscópio, um modelo de cateter de IAUP foi desenvolvido e avaliado. A passagem pela via cervical foi possível em 95,4% das fêmeas, sendo observados resultados semelhantes de taxa de prenhez e taxa de parição na comparação das fêmeas inseminadas pela IAUP (com 50 e 150 x 10^6 de espermatozóides na DI) e entre as inseminadas com três bilhões de espermatozoides pela IAT. No entanto neste estudo observou-se que a utilização de um menor número de espermatozoides na DI (10 e 25 x 10^6) com o uso da IAUP tiveram piores respostas produtivas em relação aos demais grupos de experimentação (Martinez et al. 2002). Deve-se levar em consideração que os grupos submetidos à IAUP por Martinez et al (2002), tiveram o estro sincronizado e sofreram indução da ovulação. No entanto, trabalhos que compararam o uso da IAUP em condições de campo e com estro natural observaram uma redução da taxa de parto e número de leitões nascidos (Vasquez et al., 2001; Day et al., 2003).

Assim, o desenvolvimento desta tecnologia é uma possibilidade para aplicação nas situações em que há necessidade de trabalhar com sêmen congelado-descongelado ou sêmen sexado, ou seja, em processos caracterizados por reduzir o tempo de vida das células espermáticas (Vasquez et al., 2005). Desta forma, até o momento não se recomenda o uso da IAUP na rotina produtiva das granjas comerciais.

3. Alternativas para redução do número de doses inseminantes por fêmea coberta

3.1 Protocolos tradicionais de inseminação artificial

A definição dos protocolos de IA é baseada na primeira detecção do estro. Em função da fêmea suína possuir um estro longo e existir uma grande variação individual, é difícil predizer o momento da ovulação, sendo necessário que inseminações repetidas sejam realizadas ao longo do estro para que ao menos uma delas seja realizada no momento considerado ideal (Bortolozzo et al., 2005a). Sendo assim, é comum que as fêmeas recebam três a quatro doses de sêmen durante o estro.

O intervalo considerado ideal para realização da IA em pluríparas, é de 24 horas antes da ovulação (Kemp & Soede., 1997), podendo ser estendido até 28 horas antes e 4 horas após a ovulação (Nissen et al., 1997). Sabe-se que a ovulação ocorre em média 39 horas após o início do estro em pluríparas (Heck et al., 1997) e 30 horas em nulíparas

(Uemoto, 1999). No entanto, o que se relata é uma grande variabilidade em relação ao momento da ovulação, sendo esta observação mais evidente nas leitoas.

Além da variabilidade em relação ao momento da ovulação, o número de detecções diárias de estro pode influenciar na definição do momento de realização da primeira dose. Rebanhos que realizam dois diagnósticos de estro ao dia, possuem de 5 a 8 % das porcas ovulando entre 12 e 20 horas após detecção do estro (Heck et al., 1997; Dias et al., 1999;). Em leitoas esse índice pode variar de 12,8% (Martini, 1998) a 20,6% (Uemoto, 1999). Devido a isto a primeira inseminação é realizada de 8 a 16 horas após a detecção do estro.

Nas leitoas a duração do estro é mais curta, e consequentemente, o intervalo início do estro-ovulação também é menor, o que exige que em muitas unidades seja realizada a detecção de estro duas vezes ao dia nesta categoria de fêmea e a primeira inseminação no momento da detecção do estro (Bortolozzo et al., 2005a). A definição do momento ótimo para inseminação nas leitoas ainda apresenta resultados conflitantes, sendo observadas variações de 12 (Waberski et al. 1994) a 24 horas (Uemoto, 1999) entre a inseminação e a ovulação, sem prejuízos na taxa de prenhez e número de nascidos totais ou embriões totais. Apesar disso, segundo Bortolozzo et al. (2005b), quando uma população espermática é depositada no trato genital de uma leitoa por um período superior a 16 horas antes da ovulação há, aparentemente, uma queda no desempenho reprodutivo. No entanto a realização da IA em até 24 h antes da ovulação também poderia ser considerado como intervalo ideal, sendo observado resultados satisfatórios de desempenho reprodutivo. Na prática, protocolos modernos de inseminação em leitoas com o uso de uma única detecção de estro ao dia e realização da primeira inseminação no momento da detecção do estro e as seguintes em intervalos de 24 h tem sido empregados com bons resultados de desempenho reprodutivo.

Em função da variabilidade do momento da ovulação em fêmeas suínas, a realização de múltiplas inseminações durante o estro é necessária para assegurar índices satisfatórios de desempenho reprodutivo. No entanto, ferramentas que proporcionem reduzir a variabilidade existente no intervalo início do estro e o momento da ovulação podem ser usadas como alternativas para a redução do número de doses/fêmea/estro. Isto pode ser possível através da manipulação hormonal do início do crescimento folicular e/ou do momento da ovulação permitindo a realização de inseminações em momentos predefinidos (inseminação artificial em tempo fixo).

3.2 Inseminação artificial em tempo fixo (IATF)

A utilização de protocolos hormonais permite uma melhor predição do momento da ovulação e a estruturação de protocolos IATF. Os benefícios do uso desta técnica são associados ao melhor acompanhamento do processo de IA, melhor aproveitamento da mão-de-obra, maior incremento genético, redução do número de doses/fêmea coberta e muitas vezes a eliminação do manejo de detecção de estro (Fries et al., 2010).

Em suínos, um dos protocolos mais utilizados para controle da ovulação é o uso combinado de eCG (gonadotrofina coriônica eqüina) para estimular o crescimento folicular e o hCG (gonadotrofina coriônica humana) para induzir a ovulação (Webel & Day, 1982). Estudos demonstram a eficiência da aplicação de 600 - 800 UI de eCG, no momento do desmame dos leitões ou 24 horas após, em promover o crescimento folicular suficiente para ocorrência do estro após 4 dias (Lucia et al, 1999; Candini et al., 2004; Bennett-Steward et al., 2008). A utilização de produtos que possuem ação semelhante ao LH endógeno, como o hCG e o hormônio luteinizante suíno purificado (pLH), além dos análogos do hormônio liberador de gonadotrofinas (GnRH), são utilizados na sequencia do eCG possibilitando a predição do momento da ovulação e permitindo a realização da IATF no período estabelecido para atingir a máxima fecundação (Hühn et al., 1996).

A utilização do pLH pode sincronizar a ovulação de fêmeas suínas desmamadas, por meio de um protocolo que utiliza 600UI de eCG no dia do desmame e 5mg de pLH entre 72-80h após o desmame, sendo que estudos observaram que a maioria das fêmeas ovulam aproximadamente 38 horas após a aplicação do pLH (Candini et al., 2001; Viana et al. 2002; Cassar et al. 2005). Em leitoas, o que se observa em alguns estudos (Ziecik et al., 1987; Degenstein et al., 2008; Kaeoket, 2008) é uma grande variabilidade da resposta com relação ao intervalo da aplicação hormonal e a ovulação, nos protocolos que usam o eCG seguido pela aplicação de hCG como indutor da ovulação. Isto sugere que a adequação do uso de hCG em protocolos de IATF necessita maiores estudos (Fries et al., 2010). No entanto, segundo Degenstein et al. (2008) a utilização de 600UI de eCG seguida pela aplicação de pLH 80 horas após, é capaz de induzir a ovulação em leitoas 38 horas após a aplicação de pLH.

O uso associado de eCG e um indutor da ovulação, dispensaria o manejo de detecção do estro nas porcas desmamadas, no entanto o custo dos protocolos podem inviabilizar o uso da técnica na rotina produtiva. Assim, nos últimos anos foram desenvolvidos estudos que buscaram reduzir o número de hormônios utilizados para sincronização da ovulação em fêmeas suínas. Desta forma, uma única aplicação de

indutores da ovulação considerando a detecção de estro como base para definição do momento de aplicação (Wongkaweewit et al., 2012; Zack et al, 2011; Cassar et al., 2005) ou mesmo protocolos sem considerar o estro para aplicação hormonal (Driancourt et al., 2013; Martinat-Bottè et al., 2010; Knox et al. 2014), foram estudados buscando sincronizar a ovulação para estruturação de protocolos de IATF. Estudo realizado por Wongkaweewit et al. (2012) em porcas utilizou hCG (750 UI) e GnRH (50μg – Buserilina) no momento da detecção do estro e não observaram diferença para o intervalo estro e a ovulação entre os diferentes indutores da ovulação (40.2±1.7 e 37.5±3.2 horas, respectivamente), em relação ao grupo controle onde não foi realizada aplicação hormonal (63.6±9.5 horas). De acordo com Fontana et al. (2014), a aplicação de 5 mg de pLH via intramuscular no início do estro em porcas desmamadas submetidas a uma única detecção de estro, não diferiu quanto a distribuição de frequência do momento de ovulação em relação ao grupo que não recebeu aplicação hormonal. No entanto, Fontana et al. (2014) não observaram diferenças no desempenho reprodutivo de fêmeas submetidas a uma única IATF 24 h após a aplicação de pLH em relação a fêmeas submetidas a múltiplas inseminações. De forma semelhante, Zack et al. (2011) não observaram diferenças quanto a performance reprodutiva de fêmeas submetidas a uma única inseminação 24 h após a aplicação de pLH no início do estro, em relação a fêmeas que foram inseminadas múltiplas vezes de acordo com o início do estro.

Os protocolos com aplicação de somente um indutor da ovulação sem a utilização do estro como base de definição do momento de aplicação do hormônio, utilizam análogos de GnRH como a triptorelina (Knox et al., 2014) ou buserelina (Driancourt, et al., 2013) em período predefinido em relação ao momento do desmame. Estes protocolos consideram que as fêmeas na fase de lactação apresentam o eixo reprodutivo inibido durante este período, sendo que no momento do desmame o grupo de porcas possui estágio do ciclo estral e de desenvolvimento folicular semelhantes, o que facilita a definição do protocolo de sincronização da ovulação. Esta condição, não é verificada em leitoas púberes em função das diferentes idades e momentos em que estes animais expressam o primeiro cio. Assim, a estruturação de protocolos tradicionais de IATF em leitoas, consideram protocolos hormonais com a utilização de análogos de progesterona por no mínimo 14 dias, seguido por hormônios indutores do crescimento folicular e da ovulação (Degenstein et al., 2008; Martinat-Bottè et al., 2010). Desta forma, os protocolos de leitoas representam um custo elevado que inviabiliza a utilização prática. Portanto, a pesquisa de formas alternativas de sincronização da

ovulação em leitoas se torna uma importante linha de pesquisa na definição dos protocolos hormonais e customização do uso da técnica.

Considerando que a recomendação técnica do uso de pLH (Lutropin-V®) em suínos orienta a aplicação intramuscular de 5 mg do produto, a busca pela redução da dose de pLH e a aplicação no momento da detecção do estro, proporcionaria reduzir o custo do protocolo de IATF em leitoas e porcas, otimizar o uso de doses inseminates e o manejo de inseminação nas granjas, assim como melhorar o ganho genético dos planteis.

4. Referências

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3. CAPÍTULO II – PRIMEIRO ARTIGO CIENTÍFICO

PORCINE LUTEINISING HORMONE GIVEN AT OESTRUS ONSET DOES NOT ADVANCE OVULATION IN GILTS

ARTIGO A SER SUBMETIDO PARA PUBLICAÇÃO

COMO NOTA TÉCNICA

Porcine luteinising hormone given at oestrus onset does not advance ovulation in gilts

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Abstract

This study aimed to evaluate the use of porcine luteinising hormone (pLH) given at oestrus onset in gilts to synchronise ovulation. A total of 120 gilts were assigned in three treatments: control – application of placebo by intramuscular (i.m.) route at oestrus onset; pLH2.5 - application of 2.5 mg of pLH by i.m. route at oestrus onset; pLH5 – application of 5 mg of pLH by i.m. route at oestrus onset. On average, the interval onset of oestrus to ovulation did not differ (P>0.05) among treatments (control - 28.7 ± 1.6 h; pLH2.5 - 28.2 ± 1.6 h; pLH5 - 27.5 ± 1.6 h). The frequency distribution of gilts ovulated in different moments after oestrus detection was not affected (P>0.05) by the treatment. In conclusion, the use of 2.5 mg or 5 mg of pLH given at oestrus onset in gilts by i.m. route does not advance and synchronises the interval onset of oestrus to ovulation.

Key words: ovulation induction, swine reproduction, reproductive biotechniques

Several hormonal protocols to synchronise ovulation are available for weaned sows (Martinat-Bottè et al. 2010; Zak et al., 2011; Fontana et al, 2014). However, little information is available regarding synchronised ovulation in gilts. Usually the hormonal protocols for gilts include the use of progestogens to synchronise oestrus before the application of follicle growth and ovulation inductors (Degeinstein et al. 2008; Martinat-Bottè et al. 2010). However, these protocols are laborious and expensive.

Alternative protocols to induce ovulation using pLH at oestrus onset were proposed by Zak et al. (2011) and Fontana et al. (2014) for weaned sows. In this way, this study aimed to evaluate the use of pLH given at oestrus onset in gilts to advance ovulation.

The experiment was performed in a breeding herd (6,200 sows), located in Southern Brazil, Santa Catarina State. A total of 120 gilts Camborough® (Agroceres PIC, Patos de Minas, MG, Brazil) were used. After arriving at the farm, gilts were housed in collective pens with 40 animals to perform oestrus induction once a day, using mature boars and physical contact. At first oestrus, gilts were weighed and those weighing at least 120 kg were moved to individual crates. Fence-line boar contact combined with the backpressure test was performed three times a day (8 am, 16 pm, 12 pm) starting 17 d after the first oestrus and continuing until the end of oestrus. Gilts found to be in oestrus in different shifts of oestrus detection, were assigned into three treatments: control (n=40) – application of placebo at oestrus onset by intramuscular (i.m.) route; pLH2.5 (n=40) – application of 2.5 mg of pLH (Lutropin-V® Bioniche Animal Health, Belleville, Ontario, Canada) by i.m. route at oestrus onset; pLH5 (n=40) – application of 5 mg of pLH by i.m. route at oestrus onset. Transcutaneous ultrasonography (TUS) of the ovaries was performed by a real-time ultrasonography using a convex linear transducer 5MHz (Aloka® SSD 500, Aloka Co. Ltd., Tokyo, Japan). TUS started at oestrus onset and was performed at 8 h intervals up to ovulation to determine the interval from onset of oestrus to ovulation (IOEO). Data were analysed using the Statistical Analysis System – SAS 9.1 (SAS, 2005). Results are expressed as LSmeans ± SEM and percentages according to the variable type. The continuous variables such as oestrus duration and IOEO were analysed using the MIXED procedure with a comparison of means by the Tukey-Kramer test, including the week as a random variable. The frequency distribution of IOEO in each evaluation time was analysed through Chi-squared test.

The frequency of oestrus expression was 48.3% in the morning, 26.7% in the afternoon and 25.0% at night. On average, the IOEO did not differ ($P>0.05$) among treatments (control - 28.7 ± 1.6 h; pLH2.5 - 28.2 ± 1.6 h; pLH5 - 27.5 ± 1.6 h). Differences in the oestrus duration among treatments were not observed ($P>0.05$; Table 1). The relative and cumulative frequency distribution of IOEO did not differ ($P>0.05$) among treatments in each moment of evaluation (Figure 1).

The use of 2.5 mg pLH 56 h after 600 UI of eCG given at weaning in sows was proposed by Viana et al. (2005), showing a reduction in the interval pLH application to ovulation (40.0 ± 5.9 h) in relation to control sows using only eCG at weaning (62.9 ± 14.8 h). However, in the same study, doses lower than 2.5 mg (1.25 and 0.625 mg of pLH) were not effective to advance ovulation. Similarly, Bennett-Steward et al. (2007) showed a reduction in the interval between pLH application and ovulation using 2.5 mg (39.2 ± 7.1 h) or 5 mg (41.6 ± 3.6 h) of pLH injected by the i.m. route 80 h after 600UI of eCG compared to control sows where only placebo injection was used after eCG application (55.0 ± 11.1 h). Synchronisation of ovulation in gilts was suggested by Degenstein et al. (2008) using a protocol with altrenogest, cloprostenol, eCG and 5 mg of pLH by i.m. route 80 h after eCG application. This study showed a reduction in the interval between pLH application and ovulation (43.2 ± 2.5 h) in relation to gilts that received only placebo 80 h after eCG (59.5 ± 2.5 h). Although the results cited above in weaned sows and gilts showed a positive effect of pLH after eCG on the advancement of ovulation, our observations showed that the application of 2.5 or 5 mg of pLH by the i.m. route at oestrous onset did not advance ovulation in gilts. This result corroborates those of Fontana et al. (2014), who did not observe any advance in ovulation time when 5 mg of pLH was given by the i.m. route at oestrus onset in weaned sows submitted to oestrus detection once a day. However, Ulguim et al. (2014) showed that the use of 2.5 mg of pLH by vulvar submucosal route at oestrous onset in pubertal gilts submitted to oestrus detection twice a day reduced the interval from pLH application to ovulation (32.3 ± 1.4 h) compared to gilts with no ovulation induction at oestrous onset (34.7 ± 1.4 h). In the same study, the application of 5 mg of pLH by i.m. route did not affect the interval between pLH application and ovulation (32.3 ± 1.4 h) in comparison to the use of 2.5 mg pLH by the vulvar submucosal route and control gilts. According to Ulguim et al. (2014), the frequency of sows ovulated up to 24 h after 5 mg pLH by the i.m. route (31.4%) did not differ compared to gilts that did not receive any pLH application (25.5%). Considering that the onset of LH surge can start seven hours before oestrous detection in gilts submitted to hormonal induction with eCG and pLH (Degenstein et al., 2008) and that the natural LH surge in sows can start five hours before oestrus detection (Soede et al., 1994), the application of pLH at oestrous onset could be too late to advance ovulation. Studies that showed a reduction in the interval pLH application and ovulation did not observe any effect on oestrus duration (Degenstein et al., 2008;

Ulguim et al., 2014), similar to results observed in this study. In conclusion, the use of 2.5 mg or 5 mg of pLH given at oestrus onset in gilts does not advance and synchronise the interval between the onset of oestrus and ovulation.

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Institutional Animal Care and Use Committee.

The project was approved by Institutional Animal Care of Universidade Federal do Rio Grande do Sul under the protocol number 22979.

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Table 1- Interval onset of oestrus to ovulation (h) and oestrus duration (h) of gilts submitted to application of porcine luteinising hormone (pLH) at oestrus onset using different dosages

Treatment	n	IOEO	Oestrus duration
Control	40	28.7 ± 1.6	50.0 ± 1.8
pLH2.5	40	28.2 ± 1.6	54.0 ± 1.8
pLH5	40	27.5 ± 1.6	52.2 ± 1.8

Control – application of placebo at oestrus onset; pLH2.5 – use of 2.5 mg of pLH at oestrus onset; pLH5 – use of 5 mg of pLH at oestrus onset.

IOEO – interval oestrus onset to ovulation.

Averages expressed as LSmeans ± SE.

No differences among treatments (P> 0.05).

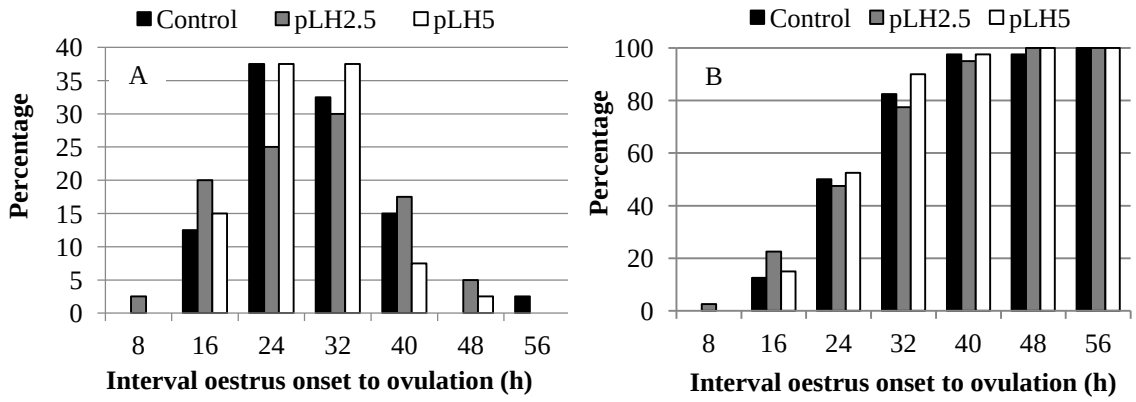

Figure 1 – Relative (A) and cumulative (B) frequency of the interval oestrus onset to ovulation in gilts submitted to application of different dosages of porcine luteinising hormone (pLH) at oestrus onset

Control – application of placebo at oestrus onset; pLH2.5 – use of 2.5 mg of pLH at oestrus onset; pLH5 – use of 5 mg of pLH at oestrus onset.

No differences among treatments in each time point of evaluation (P> 0.05).

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Use of Porcine Luteinizing Hormone at Oestrous Onset in a Protocol for Fixed-Time Artificial Insemination in Gilts

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Use of porcine luteinising hormone at oestrus onset in a protocol for fixed-time artificial insemination in gilts

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Contents

The aim of this study was to evaluate the effect of porcine luteinising hormone (pLH) given at oestrus onset in gilts, by different routes and doses, on the interval between onset of oestrus and ovulation (IOEO) and reproductive performance using a single fixed-time artificial insemination (FTAI). A total of 153 gilts were submitted to oestrus detection at 8-h intervals and assigned to three groups: control – without hormone application and inseminated at 0, 24, and 48 h after oestrus onset; VS2.5FTAI – 2.5 mg pLH by the vulvar submucosal route at oestrus onset and a single FTAI 16 h later; IM5FTAI – 5 mg pLH by the intramuscular route at oestrus onset and a single FTAI 16 h later. More VS2.5FTAI gilts (47.1%; $P<0.05$) ovulated within 24 h after oestrus onset than control gilts (25.5%) whereas IM5FTAI gilts had an intermediate percentage (31.4%; $P>0.05$). The IOEO tended to be shorter ($P=0.06$) in VS2.5FTAI (30.2 ± 1.4 h) than in control (34.7 ± 1.4 h) gilts, but there was no difference ($P>0.05$) between control and IM5FTAI (32.8 ± 1.4 h) gilts. Farrowing rate was not different ($P>0.05$) among treatments. Total born piglets (TB) was lower ($P<0.05$) in VS2.5FTAI (12.3 ± 0.4) than in control gilts (14.1 ± 0.4) whereas intermediate TB was observed in IM5FTAI gilts (13.3 ± 0.4). Due to the advancement of ovulation, reduction of the hormonal dose and the ease of application, the vulvar submucosal route would be the best option for FTAI protocols, but their negative impact on litter size remains to be elucidated. Taking into account the good fertility results obtained in IM5FTAI gilts whose ovulation was not advanced, the possibility of a single FTAI without any

hormonal treatment should be further investigated, in order to establish reliable FTAI protocols for gilts.

Keywords: reproduction; ovulation; induction; ovulation time.

Introduction

Few studies evaluating protocols for fixed-time artificial insemination (FTAI) in gilts have been performed (Martinat-Botté et al. 2010; Degenstein et al. 2008). The use of a single hormone for FTAI protocols in pubertal gilts can be difficult because they are in different phases of the oestrus cycle. This is in contrast to scenario of FTAI in weaned sows, for which several hormonal protocols to induce follicle growth and ovulation are available (Cassar et al. 2005; Martinat-Botté et al. 2010; Knox et al. 2011; Driancourt et al. 2013).

Protocols proposed for FTAI in gilts involve the use of progesterone (P4) analogue, followed or not by the use of eCG, to stimulate follicle growth, and an ovulation inductor such as GnRH, hCG, or porcine luteinising hormone – pLH (Degenstein et al. 2008; Martinat-Botté et al. 2010). According to Degenstein et al. (2008), the use of pLH is more efficient than hCG to induce ovulation after using P4 analogue and eCG in pubertal gilts. Embryo survival has not been impaired in gilts submitted to a double FTAI, after the use of P4 analogue and GnRH, compared to gilts inseminated according to the onset of oestrus (Martinat-Botté et al. 2010). The reproductive performance of gilts with double FTAI after synchronisation with P4 analogue followed by eCG and pLH application was similar to that of a control group in which multiple inseminations were performed (Degenstein et al. 2008). In weaned sows, the use of pLH at oestrus onset followed by a single FTAI 24 h later resulted in similar reproductive performance to that obtained with multiple inseminations without pLH application (Zak et al. 2011; Fontana et al. 2014).

Usually, 5 mg of pLH by the intramuscular route is the recommended dose for swine, although there are studies showing the possibility of using 2.5 mg by the intramuscular route (Viana et al. 2005; Bennett-Steward et al. 2007). On a commercial scale, the intramuscular application could require more labour, considering the difficulty of accessing the animal's neck. The vulvar submucosal route could be a practical alternative since it has been shown to be effective for farrowing induction using a single

administration of prostaglandin, allowing reduction of the dose to a quarter of the usual recommendation (Kirkwood et al. 1996; Kaeoket 2006).

The use of a single hormone application and reduction in the number of hormones used in protocols for a single or double FTAI have been considered in some studies, showing effectiveness to synchronize ovulation and reduce the number of inseminations (Martinat-Botté et al. 2010; Knox et al. 2011; Driancourt et al. 2013). This tendency in recent studies will likely facilitate the use of FTAI in the routine of commercial farms due to reduction of costs and labour with hormonal protocols.

Information about the possibility of using a single FTAI combined with induced ovulation in spontaneous oestrus is lacking for gilts. The present study aimed to evaluate the effect of different doses and routes of pLH administration at the onset of oestrus, combined with a single FTAI, on the interval between onset of oestrus and ovulation (IOEO) and reproductive performance of gilts.

Materials and methods

Animals

The study was performed during winter in a gilt development unit with an inventory of 1,200 animals located in southern Brazil – Santa Catarina State. A total of 153 Landrace × Large White Camborough 25® gilts (Agroceres PIC, Patos de Minas, MG, Brazil) were used in 3 consecutive weeks of breeding.

Housing and feeding

The gilts arrived at the farm at 161.2 ± 6.5 d of age and were housed in pens, with 20 gilts per pen. Boar stimulation to induce puberty started 10 d after they arrived, and was performed once a day using physical contact. After the first oestrus, the gilts were transferred to individual crates with a slatted floor in the breeding building, to be bred at second or third oestrus. After the last insemination, the gilts were moved to gestation facilities where they were housed in individual crates up to 50 d of pregnancy. Thereafter, pregnant gilts were transferred to a breed-to-wean farm where they were housed in groups of 12 animals (1.5 m^2/gilt). In the breeding building, they were fed 3 kg/d of a standard corn soybean diet (3,200 kcal EM/kg, 14% crude protein, and 0.7% digestible lysine) up to breeding. From insemination up to 3 days after they received 1.8 kg/day and then 2.2-2.4 kg/day were provided up to 86 days of gestation. Between 87

days of gestation until transfer to farrowing rooms, 2.6 to 2.8 kg/day were offered. *Ad libitum* access to water was provided throughout the experimental period.

Experimental design

In the breeding building, oestrus detection was carried out three times a day (7 a.m., 3 p.m., and 11 p.m.) through fenceline boar contact and back pressure test. At breeding oestrus, trios of gilts were formed according to age, weight, and number of oestrus cycles, and were assigned to the following groups: control – without hormonal application and inseminations at 0, 24, and 48 h after oestrus onset; VS2.5FTAI – use of 2.5 mg pLH (Lutropin-V, Bioniche Animal Health, Belleville, Ontario, Canada) by the vulvar submucosal route at oestrus onset and a single FTAI 16 h later; IM5FTAI – use of 5 mg pLH by the intramuscular route at oestrus onset and a single FTAI 16 h later. Trios were formed with gilts that showed oestrus in the same shift or no more than eight hours later. The time of insemination was based on results of a previous pilot study in which 80% of the gilts ovulated between 16 and 40 h after receiving 5 mg pLH at oestrus onset, by the intramuscular route (unpublished data, R.R. Ulguim).

Hormone application

For the application of 5 and 2.5 mg of pLH, 4 mL and 2 mL of Lutropin-V were used in intramuscular (5 mL syringe, 1.2 × 40 mm needle) and vulvar submucosa (3 mL syringe, 0.6 × 25 mm needle) injections, respectively. The intramuscular applications were given in the animal's neck. For the vulvar submucosal route, pLH was injected at the external vulva-cutaneous junction, at approximately 2–3 cm from the lip of the vulva. After needle introduction, the vulvar mucosa was palpated to ensure that the hormone was being properly injected.

Ultrasound evaluation

Ultrasound evaluation of ovaries was performed by a single trained technician. Transabdominal ultrasound was performed using a 5 MHz convex linear array transducer (Aloka® SSD 500, Aloka Co. Ltd., Tokyo, Japan) at 8-h intervals (8 a.m., 4 p.m., and 12 p.m.) from oestrus onset until ovulation. Ovulation was confirmed by one additional evaluation 8 h after the previous observation. The diameter of the three largest follicles was measured at each evaluation. The presence of persistent follicles

larger than 12 mm, at the last ultrasound evaluation (8 h after ovulation), was recorded. Pregnancy detection was performed by real-time ultrasonography, between 22 and 26 d after the first artificial insemination (AI).

Artificial insemination

Homospermic semen doses containing 3 billion sperm cells in a final volume of 80 mL Beltsville Thawing Solution (BTS® Minitube GmbH, Tiefenbach, Germany) were produced from ejaculates of eight boars (Agroceres PIC® 415). The semen doses were stored up to 72 h at 15–17°C. Cervical deposition of semen, in front of a mature boar, was performed by a single trained technician using a foam tip catheter (Ponta de Espuma Bretanha®, Passo Fundo, Brazil). The trios of gilts were inseminated on split-sample basis, i.e., using doses from the same ejaculate.

Statistical analysis

The data were evaluated for normality and analysed using the Statistical Analysis System – SAS 9.1 (SAS 2005). The results are expressed as least squares (LS) means ± standard error of the mean (SEM) or percentages, according to the variable type. Variables such as oestrus duration and IOEO were analysed using the MIXED procedure with comparison of means by the Tukey-Kramer test. The trio was included in the model as a random variable. Follicle size was analysed for each evaluation time through the MIXED procedure with comparison of means by the Tukey-Kramer test, including the follicle size at oestrus onset as a covariate in the model. The frequency distribution of IOEO (cumulative and at each time point of evaluation), pregnancy rate, and adjusted farrowing rate (AFR) were analysed through logistic regression using the GLIMMIX procedure. For AFR calculation, dead gilts and those culled for non-reproductive reasons were excluded. The effects of treatment, number of oestrus cycles (2 and 3), age of semen (≤24 and >24 h), and boars were included as class variables in these models. Total born piglets was analysed using the MIXED procedure, with inclusion of the fixed effects of treatment, number of oestrus cycles, boars, and age of semen, whereas the trio of gilts was included as a random effect.

Results

The gilts were inseminated at 215.8 ± 1.0 days of age, the weight of 160.2 ± 1.1 kg, and 2.1 ± 0.02 oestrus cycles, with no difference (P>0.05) among groups.

There was no effect (P>0.05) of pLH application on oestrus duration and timing of ovulation in relation to the duration of oestrus (Table 1). The use of 2.5 mg of pLH by the vulvar submucosal route tended (P=0.06) to reduce the IOEO by 4 h in comparison with control gilts (Table 1), but the use of 5 mg of pLH by the intramuscular route did not affect the IOEO (P>0.05). Furthermore, 47.1% of VS2.5FTAI gilts ovulated within 24 h compared with 25.5% in the control group (P<0.05; Figure 1A), whereas 31.4% of IM5FTAI gilts ovulated within this time. When analysed separately, frequency of gilts ovulating at each time of evaluation did not differ (P>0.05) among groups (Figure 1B).

The overall follicle size at oestrus onset was 6.0 ± 0.1 mm, with no differences among treatments (P>0.05). A greater follicle size (P<0.05) was observed in VS2.5FTAI gilts than in IM5FTAI and control gilts, at 8 and 16 h after pLH application (Figure 2). At 24 h after pLH application, gilts of both pLH groups had a greater follicle size (P<0.05) than control gilts. Persistent follicles (>12 mm) after ovulation were observed in 0, 3.9, and 3.9% of control, VS2.5FTAI, and IM5FTAI gilts (P>0.05), respectively.

Pregnancy rate and AFR did not differ among treatments (P>0.05), whereas the total number of born piglets was lower (P<0.05) in VS2.5FTAI gilts than in control gilts (Table 2). These variables were not affected by the number of oestrus cycles, boars, or semen age.

Discussion

Promising results concerning farrowing rate were obtained using protocols that included the use of pLH at oestrus onset and a single fixed-time insemination in gilts. As far as we know, this is the first study in which a reduced pLH dose (2.5 mg) was tested by the vulvar submucosal route. Its use at oestrus onset was successful in increasing the percentage of gilts ovulating within 24 h after pLH application. Although the reduction in the IOEO was slight (4 h), it is similar to that observed using more complex protocols (6 h), which involved the use of progesterone, eCG, and pLH (Degenstein et al. 2008). The higher diameter of follicles observed in VS2.5FTAI gilts

might be related to better follicle growth and consequent earliest maturation of follicles, explaining the advancement of ovulation.

Dose reduction by the vulvar submucosal route compared with the intramuscular route was described for prostaglandin analogues in swine (Kirkwood et al. 1996; Kaeoket 2006). The better response to hormonal application through the vulvar submucosal route might be attributed to the counter-current transfer of hormones in the periovarian vascular complex, allowing part of the hormone to arrive quickly to the site of action without systemic metabolisation (Stefanczyk-Krzymowska and Krzymowska 2002). Therefore, the assumption that the vulvar submucosal route could be used to reduce the pLH dose for ovulation induction in gilts was based on the effectiveness of prostaglandin in inducing farrowing (Kirkwood et al. 1996; Kaeoket 2006).

Although 5 mg pLH is the usual dosage for intramuscular application, when applied by the vulvar submucosal route, in a pilot study, it resulted in an intense oedema which was not observed when using 2.5 mg pLH. The minimal dose considered effective to induce ovulation in weaned sows (Viana et al. 2005; Bennett-Steward et al. 2007), by the intramuscular route, is 2.5 mg pLH, applied in combination with previous eCG administration. Unexpectedly, the greater dose (5 mg) used in gilts of the present study did not advance their ovulation, corroborating recent results in weaned sows (Fontana et al. 2014). Therefore, when applied at the onset of oestrus by the intramuscular route, pLH seems no to be effective for advancement of ovulation, probably because part of it undergoes systemic metabolisation before reaching the site of action.

Taking into account that the onset of LH surge can occur before oestrus onset in some gilts (Waberski et al. 1997; Degenstein et al. 2008) and that only a specific threshold of LH stimulation can be enough to initiate ovulation (Soede et al. 1994), it can be postulated that the effect of pLH may be reduced when applied at onset of oestrus. In both gilts and sows, the interval between the peak of LH concentration and ovulation is close to 30 h (Soede et al. 1994; Waberski et al. 1997; Degenstein et al. 2008). Average intervals between onset of oestrus and peak LH concentration of 8 h and 13 h have been reported for sows (Soede et al. 1994) and gilts (Waberski et al. 1997), respectively. Although these parameters are quite variable among females, avoiding a precise estimation, approximately 60% of control gilts would have already had their endogenous LH peak around onset of oestrus or even before. In this context, the effect

of pLH application on advancement and synchronisation of ovulation would be more pronounced in gilts with an expected long IOEO. Nevertheless, the percentage of control gilts showing the endogenous LH peak at the time of pLH application is probably being overestimated because their IOEO was on average shorter (35 h) than that usually (range 38 to 45 h) reported for gilts (Waberski et al. 1995, 1997; Almeida et al. 2000; Degenstein et al. 2008). As control gilts received the first insemination at the onset of oestrus, the hypothesis that their ovulation was to some extent advanced cannot be ruled out. This assumption is based on advancement of ovulation observed in gilts after the infusion of seminal plasma at the onset of oestrus (Waberski et al. 1995, 1997).

The fact that a single insemination performed at 16 h after pLH application, in IM5FTAI and VS2.5FTAI treatments, resulted in a farrowing rate similar to that for multiple inseminations may be explained by the fact that a large proportion of gilts (75.2%) from these treatments ovulated within 24 h after insemination, i.e. within an optimal interval for good fertility results (Soede et al. 1995; Bortolozzo et al. 2005). Furthermore, 12.9% of gilts were inseminated at 32 h before ovulation, which is an interval also known to give quite good fertilisation results (Kemp and Soede 1997; Fontana et al. 2014). If a single insemination at 16 h after the onset of oestrus had been used in the control group, 74.5% of the gilts would have been inseminated at 0-24 h and 11.8% at 32 h before ovulation. These observations open the possibility of a further challenging investigation concerning the fertility of gilts submitted to a single AI without any hormonal treatment.

The smaller litter size observed in VS2.5FTAI treatment was not expected given that the use of pLH as an ovulation inductor in single FTAI protocols has not been associated with reduction of litter size in gilts (RR Ulguim et al. - unpublished results) or in weaned sows (Zak et al. 2011; Fontana et al. 2014). It can be postulated that the reduction in total piglets born could be related to the insemination being performed outside the optimal interval in some gilts. Within VS2.5FTAI treatment, gilts with smaller litters (< 12 piglets) tended (P= 0.06) to ovulate earlier than those with larger litters (27.6 ± 2.1 vs. 32.9 ± 1.7 h after onset of oestrus) implying that some of them were inseminated after ovulation, which is known to impair fertility (Kemp and Soede 1997; Fontana et al. 2014). Indeed, litter size was compromised, although not statistically, in gilts inseminated after ovulation (10.6 piglets) compared to those inseminated at 0-24 h (12.3 piglets) or at 32 h (13.6 piglets) before insemination. Due to

the reduced number of VS2.5FTAI gilts inseminated after ovulation (6 gilts), it is unlikely that insemination outside the optimal interval completely explain the reduction in litter size, but other specific underlying reasons for this event could not be clarified in gilts of this group.

To our knowledge, this is the first study in which the use of only an ovulation inductor (pLH) applied at oestrus onset is evaluated in a protocol for FTAI in gilts without previous treatment with progestagens or follicular growth induction. Usually, FTAI protocols for gilts involve a combination of several hormones such as P4 analogue, eCG, and an ovulation inductor (Degenstein et al. 2008; Martinat-Botté et al. 2010), which makes the use of FTAI less feasible in production systems, due to costs and labour. Thus, the reduction in both the number and dose of hormone, as shown in the present study, can contribute to the improvement of this reproductive biotechnique in gilts. In addition, our observation in routine application of hormones showed that the application by the vulvar submucosal route reduced labour and time compared with the intramuscular route, because it is easier to access the vulva than the neck when females are housed in individual crates.

In conclusion, the results of this study show that 2.5 mg pLH applied by the vulvar submucosal route at oestrus onset slightly hastens the time of ovulation in gilts. The farrowing rate is not impaired when gilts are submitted to a single AI at 16 h after pLH administration, either by the intramuscular (5 mg) or vulvar submucosal (2.5 mg) route. Considering the reduction of the hormonal dose and the ease of application, the vulvar submucosal route would be the best option for FTAI protocols. Nevertheless, due to the smaller litter size observed following pLH application by this route, additional studies should be conducted to clarify this aspect, and to develop a FTAI protocol applicable to the routine of farms.

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Conflict of interest statement

None of the authors have any conflicts of interest to declare.

Author contributions

Rafael da Rosa Ulguim is the student (PhD level) responsible for this project. He was helped during the experimental trial by Diogo Luiz Fontana (MSc Student) and José Zacarias Rampi (undergraduate student). Prof. Mari Lourdes Bernardi provided help with the statistical analysis and drafting of the paper. Prof. Fernando Pandolfo Bortolozzo (advisor) and Prof. Ivo Wentz contributed to the design of the study and drafting of the paper.

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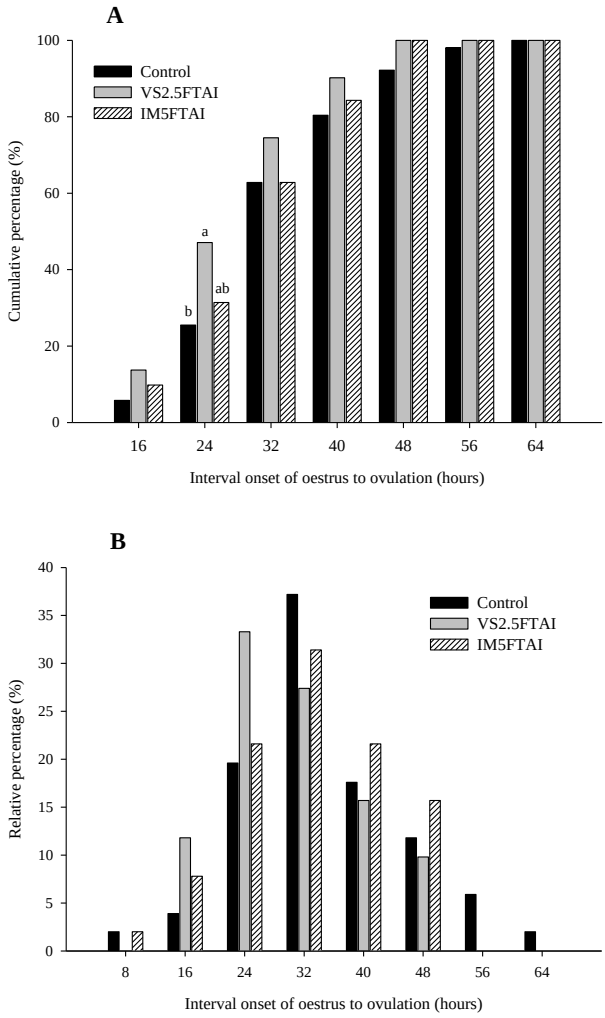

Figure 1. Cumulative (A) and relative (B) frequencies of ovulation time in gilts submitted to different protocols of porcine luteinising hormone (pLH) application. Control – without hormonal application; VS2.5FTAI – use of 2.5 mg pLH at oestrus onset by the vulvar submucosal route; IM5FTAI – use of 5 mg pLH at oestrus onset by intramuscular route.

[a–b] Significant difference among treatments ($P<0.05$).

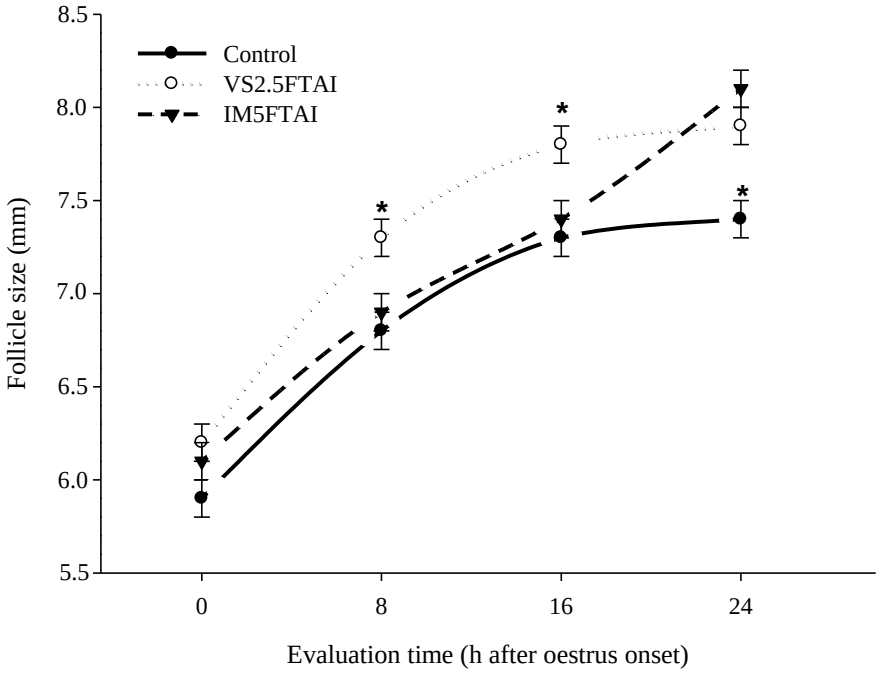

Figure 2. Follicular diameter of the three largest follicles in gilts submitted to different protocols of porcine luteinising hormone (pLH) application.

Control – without hormonal application; VS2.5FTAI – use of 2.5 mg pLH at oestrus onset by the vulvar submucosal route; IM5FTAI – use of 5 mg pLH at oestrus onset by the intramuscular route.

* Significant difference compared with the other treatments ($P<0.05$).

Table 1. Oestrus duration and interval between onset of oestrus and ovulation (IOEO) in gilts submitted to different protocols of porcine luteinising hormone (pLH) application (least squares means ± standard error of the mean).

Treatment	n	Oestrus duration (h)	IOEO (h)	% onset of estrus to ovulation[b]
Control	51	61.5 ± 2.6	34.7 ± 1.4[c]	59.5 ± 2.6
VS2.5FTAI [a]	51	60.9 ± 2.6	30.2 ± 1.4[d]	53.6 ± 2.6
IM5FTAI	51	60.5 ± 2.6	32.3 ± 1.4[cd]	55.8 ± 2.6

Control – without hormonal application; VS2.5FTAI – use of 2.5 mg pLH at oestrus onset by the vulvar submucosal route; IM5FTAI – use of 5 mg pLH at oestrus onset by the intramuscular route.

[a] One female did not ovulate.

[b] Calculated by expressing the IOEO as a proportion of the total duration of oestrus.

[c-d] Values in the column tended to be different ($P<0.06$).

Table 2. Reproductive performance of gilts according to different protocols of porcine luteinising hormone (pLH) application and artificial insemination.

Variables	Control	VS2.5FTAI	IM5FTAI
Number of inseminations	2.7 ± 0.1	1.0	1.0
Pregnancy rate, %	88.2 (45/51)	96.1 (49/51)	94.1 (48/51)
Adjusted farrowing rate, %	85.4 (41/48)	93.9 (46/49)	92.0 (46/50)
Total born piglets	14.1 ± 0.4[a]	12.3 ± 0.4[b]	13.3 ± 0.4[ab]

Control – without hormonal application and multiple inseminations according to oestrus duration (maximum of three doses per gilt); VS2.5FTAI – 2.5 mg pLH at oestrus onset by the vulvar submucosal route and a single fixed-time insemination; IM5FTAI – 5 mg pLH at oestrus onset by the intramuscular route and a single fixed-time insemination.

Results of total born piglets are presented as least squares means ± standard error of the mean.

[a–b] Values in the row are significantly different ($P<0.05$).

5. CAPÍTULO IV – TERCEIRO ARTIGO CIENTÍFICO

SINGLE FIXED-TIME ARTIFICIAL INSEMINATION IN GILTS AND WEANED SOWS USING pLH BY VULVAR SUBMUCOSAL ROUTE

ARTIGO A SER SUBMETIDO PARA PUBLICAÇÃO

Single fixed-time artificial insemination in gilts and weaned sows using pLH by vulvar submucosal route

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Abstract

This study aimed to evaluate the use of a single fixed-time artificial insemination (FTAI) in gilts and weaned sows using 2.5 mg of porcine luteinizing hormone (pLH) by vulvar submucosal route, at the onset of estrus. In experiment 1 (Exp.1), 318 pubertal gilts were assigned to two groups: Control-G - no hormonal application and inseminations (AIs) at 12, 36 and 60h after the onset of estrus; FTAI-G - use of pLH at the onset of estrus and a single FTAI 12h later. In experiment 2 (Exp. 2), 309 weaned sows were assigned to three groups: Control-S - no hormone application and AIs at 0, 24 and 48h after the onset of estrus; FTAI-NH - no hormone application and a single FTAI at 24h after the onset of estrus detection, and FTAI-pLH group - use of pLH at the onset of estrus and a single FTAI 24h later. The occurrence of semen backflow was recorded during insemination. Ultrasound evaluation was used to determine if the insemination was performed within 24 h before ovulation, considered as the optimal interval. In Exp. 1, ultrasound evaluation (12-h intervals) was carried out to determine the interval between the onset of estrus and ovulation (IOEO). Compared with Control-G, FTAI-G had shorter ($P<0.05$) duration of estrus (57.7 ± 1.1 vs 61.2 ± 1.1 h) and IOEO (36.3 ± 1.6 vs 42.3 ± 1.6 h). The adjusted farrowing rate (AFR) was lower ($P<0.05$) in FTAI-G (86.0%) compared with Control-G (93.5%) group but total piglets born (TPB) did not differ ($P>0.05$) between these groups (12.3 ± 0.2 vs 12.5 ± 0.2 piglets). Within the FTAI-G group, the AFR was lower ($P<0.05$) in the presence (73.3%) than in the absence (89.0%) of semen backflow. Also in the FTAI-G group, the insemination outside the optimal interval reduced ($P<0.05$) the TPB (10.5 ± 0.5 vs 12.9 ± 0.3 piglets) in comparison with gilts inseminated within the optimal interval. In Exp.2, there were no differences in the AFR (Control-S: 94.1%; FTAI-NH: 86.1%; FTAI-S:

88.0%) and TPB (Control-S: 12.8 ± 0.3; FTAI-NH: 12.7 ± 0.3, and FTAI-S: 12.0 ± 0.3 piglets) among treatments (P>0.05). In conclusion, 2.5 mg pLH by vulvar submucosal route reduces the farrowing rate of gilts receiving a single FTAI at 12 h after pLH application. A single FTAI performed at 24 h after the onset of estrus in weaned sows does not affect their reproductive performance regardless the use of pLH.

Keywords: ovulation induction, swine insemination, porcine luteinizing hormone

1. Introduction

The association of hormones to induce follicular growth and ovulation is commonly used in protocols for fixed-time artificial insemination (FTAI) in weaned sows (Brüssow et al., 1996; Cassar et al., 2005; Brüssow et al., 2009). After weaning sows have their estrus synchronized, at least to a certain extent, making feasible the use of a single hormone for FTAI, as it has been proposed in recent studies (Martinat-Botté et al., 2010; Knox et al., 2011; Driancourt et al., 2013; Fontana et al., 2014). The reproductive performance of weaned sows has not been compromised by the use of a single FTAI 24 h after pLH application, in comparison with multiple inseminations (Zack et al., 2011; Fontana et al., 2014).

In gilts, a previous synchronization of the estrus cycle is usually necessary before inducing ovulation in FTAI protocols. A combination of P4 analogue, eCG and an ovulation inductor is used in FTAI protocols proposed for gilts (Degenstein et al., 2008; Martinat-Botté et al., 2010). Reproductive performance of gilts submitted to double FTAI, in a protocol using the association of P4 analogue, eCG and pLH, was similar to that of gilts receiving multiple inseminations (Degenstein et al., 2008).

A single hormone to synchronize ovulation has been proposed in protocols for FTAI in gilts (Ulguim et al., 2014). The use of a single hormone without previous treatment with P4 analogue and eCG would represent a promising opportunity to intensify the use of FTAI protocols in this category.

Generally, 5 mg pLH by intramuscular route is the recommended dose to induce ovulation in swine females, although the use of 2.5 mg by intramuscular route has been proposed in some studies (Viana et al., 2005; Bennett-Steward et al., 2007). Recently, Ulguim et al. (2014) reported the effectiveness of 2.5 mg pLH, applied by vulvar submucosal route at the onset of estrus, for the advancement of ovulation in pubertal

gilts. Considering the reduction of the hormonal dose and the ease of application, the vulvar submucosal route could be a good option for FTAI protocols in both gilts and weaned sows.

By using lower doses or less hormones, costs can be reduced and labor be optimized, hence making the use of FTAI protocols feasible in the routine of the farm. The good fertility results observed by Ulguim et al. (2014), even when ovulation was not advanced, opened the possibility of investigating the effect of a single FTAI without any hormonal treatment. This study aimed to evaluate the reproductive performance of gilts and weaned sows submitted to a single FTAI after application of a reduced pLH dose at the onset of estrus, by vulvar submucosal route, in comparison with the use of multiple inseminations. In weaned sows, the reproductive performance after a single FTAI without a previous pLH application was also investigated.

2. Material and Methods

Two experiments were carried out with gilts and weaned sows in a commercial farm located at southern Brazil, in the Santa Catarina State.

2.1 Housing and feeding

The farm had an inventory of 9,000 females distributed in a gilt development unit (GDU) and breed-to-wean unit (BWU). The first experiment was carried out in the GDU where the gilts were housed in pens until the first expression of estrus and then moved to individual crates with a slatted floor in the breeding building. The pregnant gilts remained in the GDU up to 50 d of pregnancy and then were moved to BWU. The second experiment was carried out in the BWU where the weaned sows were housed in individual crates with slatted floor. In both experiments, pregnant gilts and sows were housed in collective pens of 12 animals, from 45 to 60 d of pregnancy until five days before farrowing expected date, when they were moved to farrowing rooms.

After the first estrus in gilts and weaning in sows, they were fed 3kg/d of a standard corn-soybean diet (3,200 kcal EM/kg, 14% crude protein and 0.7% digestible lysine) up to breeding. From insemination to 86 d of gestation they received a gradual increase of 2.0 to 2.4 kg/day of the same feed. From 87 d of gestation until the transfer to the farrowing rooms, they received 2.6 to 2.8 kg/day. *Ad libitum* access to water was provided throughout the experimental period.

2.2 Experimental design

2.2.1 Experiment 1: Single fixed-time insemination in gilts

In this experiment, 318 gilts (Landrace x Large White – Agroceres PIC Camborough 25; Agroceres PIC, Patos de Minas, MG, Brazil) were used, in six consecutive weeks of breeding. The gilts arrived at GDU at 155.3 ± 0.4 d of age and were housed in pens in groups of 20 gilts. Puberty induction using boar physical contact started 10 d after arrival. Thereafter, the detection of estrus was performed twice a day (7 a.m. and 7 p.m.) through fence-line boar contact and backpress test (BPT). At breeding, pairs of gilts were formed according to age, weight, number of previous estrus cycles, and assigned to the following groups: control-G – gilts without hormonal application, and artificial inseminations (AIs) performed at 12, 36 and 60 h after the onset of estrus if the gilts were still on standing estrus (maximum 3 AIs per gilt); FTAI-G – use of 2.5 mg pLH (Lutropin-V, Bioniche Animal Health, Belleville, Ontario, Canada) by vulvar submucosal route at the onset of estrus, and a single fixed-time insemination performed 12 h later.

2.2.2 Experiment 2: Single fixed-time insemination in weaned sows

In this experiment, 309 weaned sows Agroceres PIC Camborough 25 were used. The detection of estrus started at the weaning day and was performed once a day (8 a.m.) through fence-line boar contact and BPT. At estrus detection, trios of sows were formed according to parity (parity 2 to 7), lactation length (18 to 26 d), previous total born piglets (≥8), total weaned piglets (≥8) and weaning-to-estrus interval (2 to 7 d). The sows were assigned to the following groups: control-S – no hormone application and AIs performed at the onset of estrus (0 h), 24 and 48 h later if the sows were still on standing estrus (maximum 3 AIs per sow); FTAI-NH – no hormone application and a single fixed-time insemination at 24 h after the onset of estrus; FTAI-pLH – use of 2.5 mg pLH by vulvar submucosal route, at the onset of estrus, and a single fixed-time insemination 24 h later.

2.3 Hormone application

For the application of 2.5 mg of pLH, 2 mL of Lutropin-V was applied in vulvar submucosa (3 mL syringe, 0.6 x 25 mm needle). The application was performed at the external vulva-cutaneous junction, at approximately 2 to 3 cm from the lip of the vulva. After needle introduction, the vulvar mucosa was palpated to ensure that the hormone was being properly applied.

2.4 Artificial insemination

In both experiments, insemination doses were produced from ejaculates of eight boars (Agroceres PIC® 415). The ejaculates were evaluated using a computer analysis system (Sperm Vision® 3.7, Minitube GmbH, Tiefenbach, Germany) to produce homospermic doses containing 2 x 10^9 sperm cells in a final volume of 80 mL in Beltsville Thawing Solution (BTS® Minitube GmbH, Tiefenbach, Germany). The semen doses were stored up to 72 h at 15-17 °C. Semen doses stored up to 48 h were used for the treatments with a single insemination. In both experiments, the pairs or trios of females were inseminated on a split-sample basis, i.e., using doses from the same ejaculate. Cervical semen deposition was performed in gilts by a single trained technician using a foam tip catheter (Ponta de Espuma Bretanha®, Passo Fundo, Brazil) in front of a mature boar. In weaned sows, post-cervical insemination was carried out by three trained technicians using a standard foam tip catheter (Ponta de Espuma Bretanha®, Passo Fundo, Brazil) and an inner catheter (Magaplus S®, Magapor, Zaragoza, Spain). The presence or absence of semen backflow was recorded during semen deposition.

2.5 Ultrasound evaluation

The ovaries were scanned by a single trained technician using the transabdominal technique with a 5 MHz convex linear array transducer (Aloka® SSD 500, Aloka Co. Ltd., Tokyo, Japan). In Exp. 1, ultrasound evaluation was performed in all gilts, at the onset of estrus and 24 h after the last insemination, to confirm whether insemination was performed within 24 h before ovulation (optimal interval). Furthermore, a subsample of 100 gilts was evaluated at 12-h intervals to determine the interval between the onset of estrus and ovulation (IOEO). An additional evaluation was performed to confirm ovulation, 12 h after previous observation of ovulation. In Exp. 2,

a subsample of 246 weaned sows was evaluated at 24-h intervals from the onset of estrus up to 24 h after the last insemination.

2.6 Statistical analyses

The data were analyzed using the Statistical Analysis System – SAS 9.1 (SAS, 2005). The results are expressed as LSmeans ± SEM and percentages according to the variable type. The continuous variables such as the duration of estrus, IOEO, percentage of IOEO in relation to estrus duration and total number of piglets born (TPB) were analyzed using the MIXED procedure with comparison of means by the Tukey-Kramer test. The week and pairs of gilts (Exp.1) or trios of sows (Exp. 2) were included as random variables in all the models used for the analysis of continuous variables. In Exp. 1, boars and number of estruses at breeding were also tested in TPB analysis but they were not significant ($P>0.05$). In Exp. 2, parity was not significant ($P>0.05$) as a covariate whereas previous total born and weaning-to-estrus interval ($P<0.05$) were maintained in the model of TPB analysis.

The cumulative percentages of females ovulating at different intervals after pLH injection were analyzed using logistic regression models (GLIMMIX procedure), which included the fixed effect of treatment and random effects of week, and pairs of gilts (Exp. 1) or trios of sows (Exp. 2). The adjusted farrowing rate (AFR) was also analyzed using logistic regression models. For the AFR calculation, dead females and those culled for non-reproductive reasons were not included in the analysis. In Exp. 1, in addition to the effect of treatment, the model for AFR analysis included boars and number of estruses at breeding as fixed effects but they were not significant ($P>0.05$). In Exp. 2, parity and lactation length were not significant ($P>0.05$) but weaning-to-estrus interval ($P<0.05$) was maintained as a covariate in the model for AFR analysis.

In Exp. 1, reproductive performance (AFR and TPB) was also analyzed according to the optimal insemination interval (between 0 and 24 h before ovulation or not), and the occurrence or not of semen backflow during insemination.

3. Results

3.1 Single fixed-time insemination in gilts (Exp. 1)

The gilts were inseminated at 214.1 ± 0.43 d of age, weight of 157.3 ± 0.64 kg and 2.1 ± 0.02 oestrus cycles, with no difference between groups ($P>0.05$). The use of

2.5 mg pLH by vulvar submucosal route, given at the onset of estrus, reduced the duration of estrus, the IOEO and the proportion of IOEO in relation to the estrus duration ($P<0.05$) compared with Control-G gilts (Table 1). A single FTAI in gilts, 12 h after pLH injection (FTAI-G), reduced the AFR ($P<0.05$; Table 1) in comparison with the use of multiple inseminations (Control-G). However, TPB was not different between treatments ($P>0.05$).

The presence of semen backflow during insemination reduced the AFR ($P<0.05$) in FTAI-G gilts, but not in control-G gilts (Table 2). In both treatments, the occurrence of semen backflow did not affect the TPB ($P>0.05$; Table 2).

Cumulative percentages of gilts that ovulated within 36 h (70.0 vs 52.0%; $P=0.07$) tended to be different whereas there were no differences between FTAI-G and Control-G groups, respectively, at 24 h (28.0 vs 16.0%; $P=0.15$) and 48 h (96.0 vs 86.0%; $P=0.10$). The percentage of gilts that ovulated within optimal interval for insemination was greater ($P<0.01$) in control-G than in FTAI-G gilts (97.5 vs 74.2%, respectively). In FTAI-G group, fewer TPB were observed in gilts inseminated outside than those inseminated within the optimal interval ($P<0.05$; Table 3).

3.2 Single fixed-time insemination in weaned sows (Exp. 2)

Overall, the following characteristics of sows were not different ($P>0.05$) among treatments: parity (3.9 ± 0.1), lactation length (20.9 ± 0.1), previous total born piglets (13.8 ± 0.1), weaned piglets (10.8 ± 0.1), body condition score (3.4 ± 0.02) and weaning-to-estrus interval (4.1 ± 0.03). Cumulative percentages of sows that ovulated within 24 h (18.8, 12.0 and 11.0%; $P= 0.31$) and 48 h (71.8, 61.4 and 71.9%; $P= 0.24$) after the onset of estrus were similar for FTAI-pLH, FTAI-NH and Control-S, respectively. The percentage of sows that ovulated within optimal interval for insemination was greater ($P<0.0001$) in Control-S (96.3%) than in FTAI-pLH (58.8%) and FTAI-NH (53.0%) sows. The IOEO did not differ ($P>0.05$) among treatments (Table 4). There were no differences in the AFR and TPB ($P>0.05$) among treatments (Table 4).

4. Discussion

The use of pLH by vulvar submucosal route resulted in different responses between gilts and weaned sows in terms of the advancement of ovulation. Whereas the

ovulation was advanced in approximately 6 h in gilts, the pLH injection given at the onset of estrus did not advance the ovulation time in weaned sows. The advancement of the ovulation time observed in gilts following the use of a reduced pLH dose by vulvar submucosal route is in agreement with results of Ulguim et al. (2014). In the study of Fontana et al. (2014), weaned sows also had no advancement in ovulation time even receiving a higher pLH dose (5 mg), by intramuscular route, than that administered in the present study. In both gilts (Degenstein et al., 2008) and weaned sows (Soede et al., 1994), the natural LH surge can precede estrus expression by 5 to 7 h. In spite of the great variability in the relationship between the onset of estrus and maximal LH levels, the time of peak LH levels immediately precedes or is coincident with the onset of behavioral estrus in approximately two-thirds of weaned sows (Tilton et al., 1982). Considering that gilts were observed for estrus detection and ovulation time twice a day whereas weaned sows had their estrus detection performed once a day, the chance of an endogenous LH surge being already present at the moment of pLH application was higher in weaned sows than in gilts, hence reducing the possible effect of pLH on advancing the ovulation time. Therefore, the application of pLH at the onset of estrus could be too late to advance ovulation, mainly if intervals between estrus detections are long.

The feasibility of double FTAI performed in gilts that received pLH or GnRH analogue, after previous synchronization with altrenogest, has been confirmed by pregnancy rates and numbers of recovered embryos similar to those observed in gilts receiving multiple inseminations (Degenstein et al., 2008; Martinat-Botté et al., 2010). Little information is available about reproductive performance following a single FTAI in gilts. In our study, litter size was not affected but there was a reduction in farrowing rate using a single FTAI compared with the use of multiple inseminations. On the other hand, Ulguim et al. (2014) reported a reduction of litter size in gilts submitted to a similar protocol with a single FTAI 16 h after pLH injection by vulvar submucosal route at the onset of estrus.

In bovine females, administration of GnRH early in the estrus cycle may alter the subsequent luteal function (Lucy & Stevenson, 1986). Also, intrafollicular injection of LH to stimulate ovulation resulted in a suppressed subsequent luteal function in ewes (Murdoch et al., 1983). The lower farrowing rate of gilts receiving pLH could be the result of asynchronous time of LH surge (exogenous LH) with the final follicular

maturation, since fertility can be affected if follicles had not matured adequately prior to LH surge (Lucy & Stevenson, 1986). Although this hypothesis cannot be ruled out, the main explanation for the reduced AFR in FTAI-G gilts of the present study seems to be the occurrence of semen backflow during insemination, which reduced the AFR in 16%. According to Steverink et al. (1998), semen backflow during and after insemination is a normal process in swine. However, the loss of semen could reduce the number of sperm available for fertilization. In weaned sows submitted to a single post-cervical insemination 24 h after pLH injection, Fontana et al. (2014) also observed that semen backflow reduced the AFR and TPB. Thus, females submitted to protocols of single insemination and that present semen backflow during semen deposition should be re-inseminated to prevent poor reproductive performance, as previously suggested by Fontana et al. (2014).

The protocols of a single FTAI are defined considering that insemination until 24 h before ovulation is the optimal interval to obtain good fertilization results (Kemp & Soede, 1997; Bortolozzo et al., 2005). In swine females submitted to multiple inseminations is more common that they receive at least one insemination within the optimal insemination-ovulation interval. This was confirmed in the present study in which the percentage of females that ovulated within optimal interval for insemination was greater in both gilts and sows receiving multiple inseminations than in those single-inseminated. The smaller litter size of FTAI-G inseminated outside the optimal interval is in agreement with previous reports that fertilization rate (Soede et al. 1995) or litter size (Bortolozzo et al., 2005) are reduced in females inseminated at >24 h before ovulation.

The similar reproductive performance (farrowing rate and TPB) between weaned sows single inseminated, after receiving 2.5 mg pLH by vulvar submucosal route, and those receiving multiple inseminations corroborates previous results of studies in which single (Zack et al., 2011; Fontana et al., 2014) or double (Stewart et al., 2010; Knox et al., 2011; Driancourt et al., 2013) fixed-time inseminations were used after synchronization of ovulation. Unlike the studies previously cited, our Exp. 2 included a negative control treatment using a single FTAI without hormone application. Surprisingly, a single FTAI 24 h after the onset of estrus in weaned sows submitted to estrus detection once a day resulted in similar reproductive performance compared with the use of multiple inseminations, regardless of pLH use. Thus, it seems that pLH

application in the FTAI protocol for weaned sows, used in the present study, did not contribute to improve the reproductive performance when compared to a protocol without hormonal induction. Although FTAI sows without hormonal treatment had a reduction of 8% in AFR in comparison to Control-S, this difference was not statistically significant. Therefore, within this promising context of avoiding the use of hormones, it would be important to perform additional studies to evaluate the use of a single FTAI without previous hormone application to induce ovulation.

5. Conclusion

The application of 2.5 mg of pLH by vulvar submucosal route, at estrous onset, advances the ovulation time in gilts submitted to estrus detection twice a day. However, the ovulation time is not advanced in weaned sows submitted to estrus detection once a day. The use of a single fixed-time insemination at 12 h after pLH injection reduces the farrowing rate of gilts. A single fixed-time insemination performed in weaned sows at 24 h after the onset of estrus does not affect their reproductive performance regardless of the pLH use.

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Table 1 – Reproductive performance of gilts using pLH at the onset of estrus to synchronize ovulation followed by a single fixed-time insemination (Exp. 1)

Variables	Control-G	FTAI-G	P-value
Number of gilts	159	159	-
Estrus duration, h	61.2 ± 1.1	57.7 ± 1.1	0.02
IOEO[a], h	42.3 ± 1.6	36.3 ± 1.6	0.01
Ovulation/estrus duration[b], %	71.6 ± 2.5	64.2 ± 2.5	0.03
Number of inseminations	2.3 ± 0.1	1	-
Adjusted farrowing rate[c], %	93.5 (145/155)	86.0 (135/157)	0.03
Total piglets born	12.5 ± 0.2	12.3 ± 0.2	0.51

Values are expressed as Least Squares Means ± Standard error of mean.

Control-G: gilts did not receive pLH and inseminations were performed at 12, 36 and 60 h after the onset of estrus if they were on standing estrus.

FTAI-G: use of 2.5 mg pLH at the onset of estrus by vulvar submucosal route and a single insemination 12 h later.

[a]IOEO= The interval between onset of estrus and ovulation was evaluated in a subsample of 50 gilts per treatment, at 12-h intervals.

[b]Calculated by expressing the IOEO as a proportion of the total duration of estrus.

[c] The Adjusted farrowing rate was analyzed using a Logistic regression model.

Table 2 – Reproductive performance according to the occurrence of semen backflow during insemination in gilts subjected to different insemination protocols (Exp. 1)

Variable	Treatment	Semen backflow[a]		P-value
		Absence	Presence	
Adjusted farrowing rate[b], % (n/n)	Control-G	95.2 (100/105)	90.0 (45/50)	0.19
	FTAI-G	89.0 (113/127)	73.3 (22/30)	0.02
Total piglets born[c]	Control-G	12.4 ± 0.3	12.9 ± 0.4	0.34
	FTAI-G	12.3 ± 0.3	12.3 ± 0.7	0.94

Control-G: gilts did not receive pLH and inseminations were performed at 12, 36 and 60 h after onset of estrus if they were on standing oestrus.

FTAI-G: use of 2.5 mg pLH at onset of estrus by vulvar submucosal route and a single insemination 12 h later.

[a] The presence of backflow during insemination in at least one of inseminations performed in each gilt is being considered in this analysis.

[b] The adjusted farrowing rate was analyzed using a Logistic regression model.

[c] Values are expressed as Least Squares Means ± Standard error of mean.

Table 3 – Reproductive performance of gilts according to the insemination within the optimal interval - between 0 and 24 h before ovulation (Exp. 1)

Variable	Treatment	Interval insemination-ovulation		P-value
		Optimal	Not optimal	
AFR[a] ,% (n/n)	Control-G	94.7 (143/151)	50.0 (2/4)	0.01
	FTAI-G	86.2 (100/116)	85.4 (35/41)	0.89
Total piglets born[b]	Control-G	12.5 ± 0.2	13.0 ± 1.9	0.81
	FTAI-G	12.9 ± 0.3	10.5 ± 0.5	<0.0001

Control-G: gilts did not receive pLH and inseminations were performed at 12, 36 and 60 h after the onset of estrus if they were on standing estrus.

FTAI-G: use of 2.5 mg pLH at the onset of estrus by vulvar submucosal route and a single insemination 12 h later.

[a] The adjusted farrowing rate (AFR) was analyzed using a Logistic regression model.

[b] Values are expressed as Least Squares Means ± Standard error of mean.

Table 4 - Reproductive performance of weaned sows using pLH at onset of estrus and a single fixed-time artificial insemination (Exp. 2)

Variable	Control-S	FTAI-NH	FTAI-pLH	P-value
IOEO[ac], h	51.0 ± 2.5	51.3 ± 2.5	47.8 ± 2.5	0.17
Number of inseminations	2.5 ± 0.1	1	1	-
Adjusted Farrowing Rate[b], % (n/n)	94.1 (96/102)	86.1 (87/101)	88.0 (88/100)	0.19
Total piglets born[c]	12.8 ± 0.3	12.7 ± 0.3	12.0 ± 0.3	0.12

Control-S: sows did not receive pLH and inseminations were performed at 0, 24 and 48 h after the onset of estrus if they were on standing oestrus.

FTAI-NH: sows did not receive pLH and a single insemination was performed at 24 h after the onset of estrus.

FTAI-pLH: pLH was administered at the onset of estrus by vulvar submucosal route and a single insemination was performed 24 h later.

[a] IOEO= The interval between onset of oestrus and ovulation was evaluated at 24-h intervals, in a subsample of 250 gilts.

[b] The adjusted farrowing rate was analyzed using a logistic regression model.

[c] Values are expressed as Least Squares Means ± Standard error of mean.

6. CONSIDERAÇÕES FINAIS

Atualmente tem-se observado um incremento no número de pesquisas que buscam estruturar protocolos hormonais para predizer o momento da ovulação e predefinir o momento para realização da inseminação em fêmeas suínas. Neste sentido, a inseminação em tempo fixo surge como uma ferramenta promissora no cenário da reprodução em suínos com o objetivo de otimizar o uso de machos geneticamente superiores, incrementar o ganho genético e reduzir a mão de obra envolvida nos programas de inseminação em suínos.

Os protocolos mais conservadores de sincronização da ovulação preconizam a utilização de combinações hormonais com o uso de indutores de crescimento folicular e da ovulação. O uso de vários hormônios representa um maior custo do protocolo que pode inviabilizar a utilização da técnica na rotina das granjas. Neste sentido, tem-se observado nos últimos anos a estruturação de protocolos hormonais que utilizam somente indutores de ovuluação em momento predefinido. Em fêmeas desmamadas o uso destes protocolos são facilitados em função destes animais possuírem o ciclo estral sincronizado ao desmame. Nas leitoas esta condição não acontece, sendo que nesta categoria a utilização de hormônios para sincronização do estro seguido pelo uso de hormônios para a sincronização da ovulação são necessários. Assim, principalmente nas leitoas a utilização do estro como ponto inicial de definição da aplicação do hormônio para sincronização da ovulação pode ser considerado uma alternativa. Da mesma forma, em fêmeas desmamadas a utilização de um único hormônio no início do estro pode representar também uma oportunidade de redução do custo para sincronização da ovulação e realização de uma única inseminação em tempo fixo.

Os resultados dos estudos realizados nesta tese mostram que a aplicação de 2,5 mg de pLH no início do estro em leitoas antecipou o intervalo inicio do estro e a ovulação quando o hormônio foi aplicado pela via submucosa vulvar, comparado as leitoas que não receberam o hormônio. No entanto, em fêmeas desmamadas a utilização de pLH por via submucosa vulvar não antecipou o momento da ovulação. O uso de 5 mg de pLH aplicado no ínicio do estro por via intramuscular não proporcionou a antecipação da ovulação. Embora uma antecipação no momento da ovulação tenha sido observado em leitoas, sugere-se que a aplicação do pLH no início do estro pode ser

tardia para se alcançar resultados mais expressivos em termos de antecipação da ovulação, considerando que o pico de LH pode acontecer antes da detecção do estro.

O uso de uma única inseminação em tempo fixo afetou a performance reprodutiva de leitoas em relação àquelas que receberam múltiplas inseminações. No entanto nas fêmeas desmamadas, o uso de uma única inseminação não assegurou diferença nos índices reprodutivos em relação ao grupo controle com o uso de múltiplas inseminações, independentemente do uso de pLH. Assim, tanto em leitoas quanto em porcas desmamadas, pesquisas adicionais que contemplem a realização de uma única inseminação em momento predefinido sem a utilização de indutores da ovulação no momento do estro são importantes para avaliar a efetividade no uso destes hormônios na indução da ovulação.