UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

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IMPACTO DO DIÂMETRO DO FOLÍCULO DOMINANTE NO MOMENTO DA RETIRADA DO DISPOSITIVO INTRAVAGINAL DE PROGESTERONA EM UM PROGRAMA DE IATF

Santa Maria, RS

2018

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-graduação em Medicina Veterinária, Área de concentração em Sanidade e Reprodução Animal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Medicina Veterinária**.

Orientador: Dra. Mara Iolanda Batistella Rubin

Santa Maria, RS
2018

Boligon de Araujo, Laurence
Impacto do diâmetro do folículo dominante no momento da retirada do dispositivo intravaginal de progesterona em um programa de IATF / Laurence Boligon de Araujo.- 2018.
33 p.; 30 cm

Orientadora: Mara Iolanda Batistella Rubiun
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Medicina Veterinária, RS, 2018

1. Inseminação Artificial 2. Folículo dominante 3. Prenhez 4. IATF 5. Braford I. Iolanda Batistella Rubiun, Mara II. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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11 de agosto de 2018:

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Santa Maria, RS
2018

RESUMO

IMPACTO DO DIÂMETRO DO FOLÍCULO DOMINANTE NO MOMENTO DA RETIRADA DO DISPOSITIVO INTRAVAGINAL DE PROGESTERONA EM UM PROGRAMA DE IATF

AUTOR: Laurence Boligon de Araujo
ORIENTADOR: Mara Iolanda Batistella Rubin

O diâmetro do folículo pré-ovulatório, no momento da inseminação artificial, tem impacto significativo sob a taxa de prenhez de vacas de corte submetidas a programas de sincronização de cio e ovulação. O objetivo deste estudo foi verificar o efeito do diâmetro do folículo dominante em um protocolo IATF com estrógeno e progesterona. Neste estudo foram utilizadas 227 novilhas de 2 anos e 143 vacas lactantes da raça Braford. Na sincronização dos animais aplicou-se benzoato de estradiol e um dispositivo intravaginal de progesterona (Dia 0). No dia de retirada do dispositivo de progesterona (Dia 8) foram aplicados via IM: Prostaglandina F2α, cipionato de estradiol e gonadotrofina coriônica equina (eCG). A inseminação artificial (IA) efetuou-se 48 horas após a remoção do dispositivo de progesterona (Dia 10). Adicionalmente, as novilhas receberam no Dia 0 uma dose de prostaglandina F2α. Exames de ultrassonografia foram realizados nos Dias 0, 8 10 e 40 do protocolo para verificar a presença de CL, diâmetro folicular e realizar o diagnóstico de gestação. O estudo foi conduzido em uma fazenda comercial sediada na fronteira oeste do estado do Rio Grande do Sul. A distribuição do diâmetro dos folículos no Dia 8 foi didaticamente dividida em sete grupos (≤7; 7,1 a 8; 8,1 a 9; 9,1 a 10; 10,1 a 11; 11,1 a 12 e >12 milímetros de diâmetro). Cerca de 25% das vacas e novilhas estavam no grupo ≤7 mm, com taxa prenhez de 5,88 e 23,08%, sendo menor ($P<0,05$) que as demais categorias de folículos nas vacas.No entanto, as novilhas do grupo >12 mm apresentam taxa de prenhez semelhante à do grupo ≤7 mm e abaixo ($P<0,05$) dos demais grupos de folículos. Adicionalmente, o conhecimento científico obtido neste estudo revela que o exame de ultrassonografia é uma alternativa para decisões individuais conforme o diâmetro do folículo dominante na retirada do dispositivo de progesterona em protocolos de IATF. Assim, proporciona melhor eficiência reprodutiva em função da capacidade do folículo pré-ovulatório em responder ao hormônio luteinizante e ovular o mais próximo da inseminação artificial.

Palavras-chave: Inseminação artificial, folículo dominante, prenhez, IATF, Braford.

ABSTRACT

DOMINANT FOLLICLE SIZE AT PROGESTERONE-RELEASING DEVICE WITHDRAWAL DISTRIBUTION AND IT IMPACT ON A FTAI PROGRAM

AUTHOR: Laurence Boligon de Araujo
ADVISOR: Mara Iolanda Batistella Rubin

The preovulatory follicle size at the time of artificial insemination has a significant impact on the pregnancy rate of beef cattle submitted to estrus synchronization programs. The objective of this study was to verify the effect of dominant follicle diameter on pregnancy rate in an FTAI protocol based on estradiol and progesterone. For the development of the study 227 heifers with two years old and 143 Braford suckling cows were used. Heifers and cows was placed an intravaginal progesterone device, new device was use for cows and pre-used for heifers, and estradiol benzoate (im) on Day 0. Prostaglandin, estradiol cipionate and eCG was injected (im) at the time of progesterone device removal on Day 8, FTAI was proceeded 48 hours after device removal on Day 10. In addition, heifers received (im) prostaglandin on Day 0. Ultrasonography was performed on days 0, 8, 10 and 40 to check the presence of CL, follicle size and pregnancy rates. The study was carried out in a private farm, located in the western border of Rio Grande do Sul, Brazil. The distribution of the follicular size on day 8 was didactic divided in seven groups (≤7, 7.1 to 8, 8.1 to 9, 9.1 to 10, 10.1 to 11, 11.1 to 12 and >12 mm of diameter). Around 25% of the cows and heifers were in the ≤7 mm group with 5.88 and 23.08% of pregnancy rate, smaller ($P < 0.05$) than the other groups of follicles in cows. However, heifers of the group > 12 mm the pregnancy rates are similar to the group ≤7 mm and below ($P < 0.05$) of the other groups of follicles sizes. Additionally, the scientific knowledge obtained in this study provides that ultrasound exam an alternative for individual decisions according to the dominant follicle size at the progesterone device withdrawal on FTAI protocols. Enhanced reproductive efficiency for promote the preovulatory follicle to respond to the luteinizing hormone and to ovulate close the time of artificial insemination.

Keywords: Artificial insemination, dominant follicle size, pregnancy rates, FTAI, Braford.

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1 INTRODUÇÃO

A aplicação da técnica de inseminação artificial (IA) está difundida no mundo inteiro e bilhões de doses de sêmen são produzidas por ano, tanto para a indústria de bovinos de corte como de leite (THIBIER e WAGNER, 2002). Os programas de inseminação artificial em tempo fixo (IATF) vêm causando impacto positivo sobre o fornecimento de comida no mundo por possibilitar melhoramento genético e aumentar a eficiência reprodutiva dos rebanhos (HANSEN, 2014). Os protocolos de IATF baseados na aplicação de benzoato de estradiol (BE) e no uso de progesterona por sete, oito ou nove dias através de um dispositivo intravaginal liberador do hormônio vem sendo amplamente utilizados na América do Sul em bovinos de corte (BÓ et al., 2013), juntamente com a aplicação de cipionato de estradiol (CE) para reduzir o número de manejos e possibilitar a sincronização da ovulação (MENEGHETTI et al., 2009; SILVA et al., 2018). O uso da gonadotrofina coriônica equina (eCG) tem como objetivo aumentar o crescimento do folículo dominante, a taxa de ovulação, o diâmetro do corpo lúteo (CL) e a taxa de prenhez (BÓ et al., 2016).

Desde o desenvolvimento dos programas de IATF a taxa de prenhez por inseminação artificial têm sido próxima a 50% (PFEIFER et al., 2015). Por isso, uma série de estudos vem investigando o efeito do diâmetro do folículo pré-ovulatório (FPO), demonstrando que o diâmetro é um forte indicador de fertilidade, possivelmente melhor que a concentração sérica de estrogênio e a demonstração prévia de estro no momento da IA (VASCONCELOS et al., 2001; PERRY et al., 2005; PERRY et al., 2014). Folículos pré-ovulatorios de 10,8 a 15,6 milímetros de diâmetro resultaram nas melhores taxas de prenhez em novilhas (PERRY et al., 2007) quando comparados a diâmetros maiores e/ou menores que estes. Dadarwal et al. (2013) demonstraram que o pró-estro curto em fêmeas influencia negativamente a taxa de prenhez. Por outro lado, Bó et al. (2016) ao prolongar o pró-estro em protocolos de IATF e Pfeifer et al. (2015) ao ajustarem o tempo deste período conforme o diâmetro do FPO, obtiveram um aumento do diâmetro do FPO no momento da IA, possibilitando melhoria na taxa de ovulação e maior taxa de prenhez quando comparado aos protocolos tradicionais. No entanto, não se tem informações sobre a distribuição do diâmetro do folículo dominante no momento da retirada do dispositivo liberador de progesterona e o seu impacto nos programas de IATF. A hipótese do presente estudo é que o diâmetro do folículo dominante no momento da retirada do dispositivo intravaginal liberador de progesterona tem efeito sobre a taxa de prenhez em protocolos de IATF com base em estrógenos e progesterona. O objetivo desse estudo foi determinar o diâmetro do folículo dominante no dia da retirada do dispositivo de

progesterona e seu efeito sobre a taxa de prenhez em um programa de IATF em vacas lactantes e novilhas de corte da raça Braford.

2 REVISÃO BIBLIOGRÁFICA

2.1 Inseminação Artificial

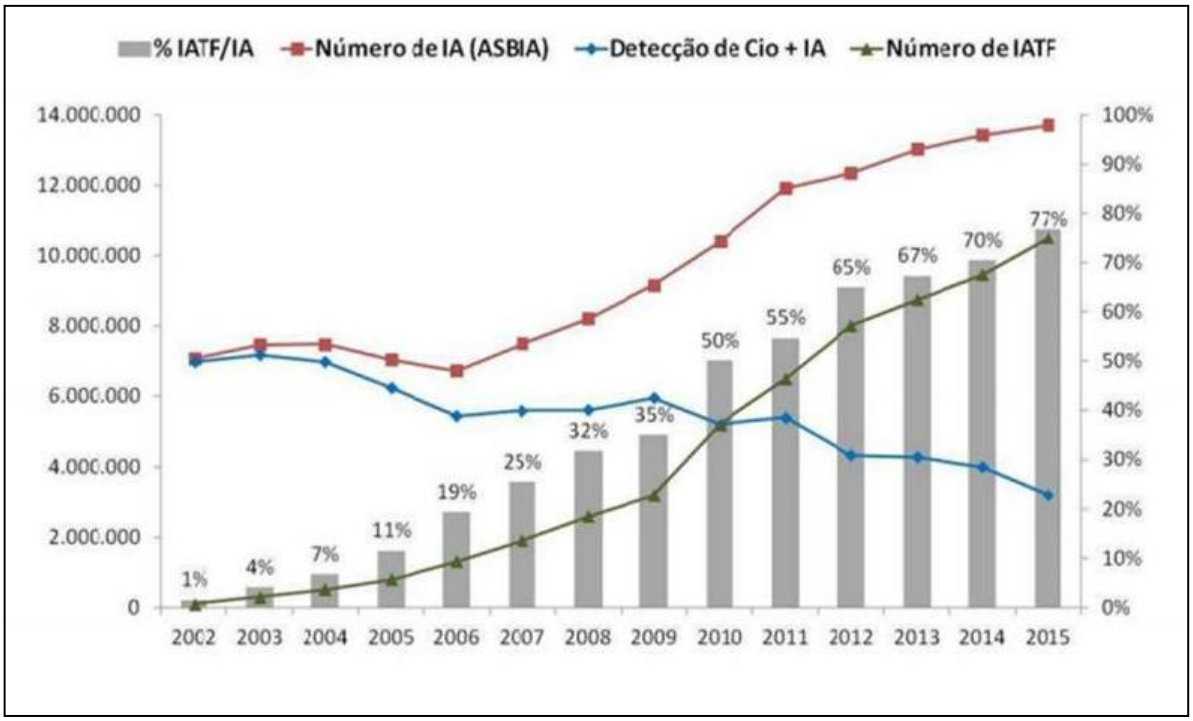
A inseminação artificial é uma biotecnologia utilizada pelos os pecuaristas para melhorar o progresso genético e controlar doenças venéreas nos rebanhos de corte (BARUSELLI et al., 2018). A alta eficiência reprodutiva é um requisito fundamental para garantir uma produção pecuária sustentável e para o produtor de carne bovina obter retornos econômicos satisfatórios. Por isso, lançar mão de programas reprodutivos na rotina da propriedade é uma maneira de otimizar os resultados reprodutivos e a rentabilidade do segmento (THIBIER & WAGNER, 2002; LAMB & MERCADANTE, 2016). Os diferentes programas de inseminação artificial apresentam muitas vantagens para o sistema das propriedades de corte quando comparados a monta natural (LIMA et al., 2010; LAMB & MERCADANTE, 2016). Algumas destas vantagens são identificadas como a possibilidade de utilizar a genética de touros que não estão presentes na fazenda e que, por vezes, são de raças não adaptadas as condições ambientais da propriedade, mas que permitem a produção de terneiros mestiços para maximizar ganhos e atingir diferentes mercados, aumentar a uniformidade do rebanho e acelerar o ganho genético, resultando em progênies mais produtivas e lucrativas (RODGERS et al., 2015; BARUSELLI et al., 2017), além, de evitar a transmissão de doenças venéreas (VISHWANATH, 2003).

Os avanços na área de biotecnologia da reprodução possibilitaram a compreensão da dinâmica do ciclo estral bovino, o que resultou no desenvolvimento de protocolos para a manipulação do ciclo estral e controle da ovulação (RODGERS et al., 2015). Estes protocolos de sincronização do estro (SE) ou de IATF apresentam taxas de prenhez semelhantes aos protocolos que necessitam da detecção de estro (LARSON et al., 2006). Porém, o uso da SE e da IATF tem o potencial para influenciar a eficiência econômica das propriedades de criação de terneiros (SPROTT, 1999).

Os programas de inseminação artificial em tempo fixo estão sendo utilizados com sucesso tanto em vacas Bos taurus taurus como em Bos taurus indicus. Esses programas oferecem uma abordagem organizada para melhorar o uso da inseminação artificial, bem como para aprimorar a eficiência reprodutiva em rebanhos bovinos (PERRY et. al., 2005). Entre as técnicas de inseminação artificial, a técnica em tempo fixo é utilizada mundialmente. Nos últimos anos, o tratamento com estrógenos e progestágenos tem sido empregado cada vez mais para programas de sincronização do estro em vacas de leite e corte (COLAZO et al.,

1999; KASTELIC et al., 1996; MACMILLAN & BURKE, 1996; MARTINEZ et al., 2002). Esta técnica possibilita que as vacas sejam inseminadas e concebam no início da estação de monta, diminuindo o período de serviço e aumentando a eficiência reprodutiva do rebanho (ODDE, 1990; SÁ FILHO et al., 2009). Desta forma, pela crescente demanda da inseminação artificial no Brasil (Figura 1) e no mundo, é preciso que continue o investimento em estudos que busquem uma melhor e eficiência desses programas e por consequência uma maior rentabilidade para propriedades.

Figura 1. Crescimento do uso das biotécnicas de IATF/IA em percentual e número de IA, detecção de cio + IA e IATF no Brasil de 2002 a 2015.



Fonte: BARUSELLI, 2016

2.2 Diâmetro folicular em protocolos de IATF

Procedimentos que controlam o tempo de ovulação em bovinos são fundamentais no avanço do uso de tecnologias de reprodução assistida. Para bovinos, vários protocolos são efetivos no controle do ciclo estral e reduzem o tempo necessário para detectar o estro (ODDE, 1990; LUCY et al., 2001), porém o momento da ovulação é impreciso, o que dificulta a inseminação artificial dos animais em um tempo fixo (PERRY et al., 2005). Os folículos em bovinos atingem capacidade ovulatória com 10 mm de diâmetro. No entanto,

uma dose maior de hormônio luteinizante é necessária para induzir a ovulação de um folículo de 10 mm do que a ovulação de folículos maiores (SARTORI et al., 2001).

Fatores como estados de condição corporal (ECC), dias pós-parto, diâmetro do FPO, idade e raça das fêmeas, sêmen, manejo e mão de obra são relatados por influenciar o sobre a taxa de prenhez em protocolos de IATF em gado de corte (PERRY et al., 2005, 2007). Porém, o diâmetro do folículo ovulatório possivelmente é o fator que mais influencia na sincronização do tempo de ovulação em novilhas (PERRY et al., 2007) e vacas lactantes de corte (PERRY et al., 2005). Para mensurar o diâmetro do FPO é utilizado o ultrassom em tempo real (B-mode), sendo este um método rápido e preciso (FRICKE, 2002). O mesmo é utilizado para o diagnóstico precoce de gestação que pode ser efetuado do 26º ao 35º dias de gestação (RACEWICZ et al., 2016).

Com o objetivo de diminuir a mortalidade embrionária no período crítico da gestação e aumentar a taxa de prenhez em programas de inseminação artificial, Binelli et al. (2001) indicaram uma série de estratégias que visam tanto diminuir a atividade luteolítica uterina, quanto aumentar o efeito antiluteolítico provocado pelo concepto. Estas estratégias incluíam: aumentar o diâmetro do folículo pré-ovulatório para gerar um corpo lúteo (CL) maior; aumentar a taxa de crescimento do CL; incrementar o nível de progesterona durante a fase luteínica; diminuir o efeito de um folículo dominante (FD) durante o período crítico. Todavia, é preciso quantificar o impacto do diâmetro folicular sobre a taxa de prenhez, tanto no dia da inseminação quanto dois dias antes, no momento da retirada do dispositivo de progesterona.

Na década passada, o estudo que induziu a ovulação de vacas de leite com folículos <11,5 mm resultou em CL menor e segregaram menor concentração de progesterona do que vacas ovulando folículos maiores (VASCONCELOS et al., 2001), ao passo que, vacas com maior folículo pré-ovulatório desenvolveram um corpo lúteo de maior diâmetro, portanto, produziram mais progesterona, favorecendo o desenvolvimento embrionário (RIBEIRO et al., 2016). Ambas pesquisas acrescentaram importantes informações, no entanto não se sabe o tamanho médio do diâmetro dos folículos dominantes no momento da retirada do dispositivo liberador de progesterona em programas de IATF e seu impacto sobre a taxa de prenhez.

3 CAPÍTULO I

Dominant follicle size at progesterone-releasing device withdrawal distribution and it impact on FTAI program

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Artigo a ser submetido ao periódico *Animal Reproduction*

Agosto, 2018

Dominant follicle size at progesterone-releasing device withdrawal distribution and it impact on FTAI program

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Article type: Applied

Abstract

The objective of this study was to verify the distribution of dominant follicle size at the progesterone-releasing device withdrawal and it impact on pregnancy rate in an FTAI protocol based on estradiol and progesterone. For the development of the study, 227 heifers with 2 years old and 143 Braford suckling cows were used. Heifers and cows was placed a intravaginal progesterone device, new device was use for cows and pre-used for heifers, and 2 mg estradiol benzoate on Day 0, 0,5mg of prostaglandin, 1mg estradiol cipionate and 300 IU eCG at the time of intravaginal device removal on Day 8, FTAI was proceeded 48 hours after device removal on Day 10. In addition, heifers received 0,5mg of prostaglandin on Day 0. Ultrasonography was performed on Days 0, 8, 10 and 30 to check the presence of CL, follicle size and pregnancy rates. The study was carried out in a private farm, located in the western border of Rio Grande do Sul, Brazil. The distribution of the follicular size on day 8 was didactic divided in seven groups (≤7, 7.1 to 8, 8.1 to 9, 9.1 to 10, 10.1 to 11, 11.1 to 12 and >12 mm of diameter). Around 25% of the cows and heifers were in the ≤7 mm group with 5.88 and 23.08% of pregnancy rate, smaller ($P < 0.05$) than the other groups of follicles in cows. However, heifers of the group > 12 mm the pregnancy rates are similar to the group ≤7 mm and below ($P < 0.05$) of the other groups of follicles sizes. Additionally, the scientific knowledge obtained in this study provides that ultrasound exam an alternative for individual decisions according to the dominant follicle size at the progesterone device withdrawal on FTAI protocols. Enhanced reproductive efficiency for promote the preovulatory follicle to respond to the luteinizing hormone and to ovulate close the time of artificial insemination.

Keywords: Artificial insemination, dominant follicle size, pregnancy rates, FTAI, Braford.

Introduction

Artificial insemination (AI) appliance is spread throughout the world and several million doses of semen were produced per year for a dairy and beef cattle industry (Thibier and Wagner, 2002). Fixed timed-AI and AI programs have critical impact in the world food throught genetic improvement and reproductive efficiency (reviewed by Hansen, 2014). Estradiol and progesterone based protocols to 7, 8 or 9 days with intravaginal progestin-releasing device have been used widely in South America (Bó et al., 2013) with estradiol cipionate (EC) for reduce the animal handling and further synchronizing ovulation (Meneghetti et al., 2009; Silva et al., 2018) and equine chorionic gonadotropin (eCG) for improve preovulatory follicle growth, ovulation, diameter of the corpus luteum (CL) and its progesterone production and pregnancy rates in beef cattle (reviewed by Bó et al., 2016).

Since the beginning of TAI programs development the pregnancy rates per AI (P/AI) are around 50% (Pfeifer et al., 2015). For this reason, several studies were investigate the effect of pre ovulatory follicle (POF) size and have been shown as a strong indicator of fertility, better than serum concentration of estradiol at time of AI or expression of estrus (Vasconcelos et al., 2001; Perry et al., 2005; Perry et al., 2014). In 2007, Perry et al. reported that heifers POF between 10.8 to 15.6 mm result in greater pregnancy rates. Dadarwal et al. (2013) have been demonstrated that short proestrous decrease P/AI. On the other hand, Bó et al. (2016) prolonging the proestrous in FTAI protocols and Pfeifer et al. (2015) delaying AI according on the diameter of the preovulatory follicle, called TAI in blocks, demonstrated an increase the diameter of the POF at the time of AI and possibly a greater ovulation rate and greater P/AI. However, the distribution of follicular diameter at the time of progesterone device withdrawal and its impact on TAI programs has not been reported. The hypotheses of this study are that dominant follicle size at time of progesterone device withdrawal impact on pregnancy rates in estradiol-based FTAI. The objective of this study was determine the effect of dominant follicle size at intravaginal progesterone device withdrawal (Day 8) on pregnancy rate in estradiol-based FTAI in Braford (5/8 Bos taurus x 3/8 Bos indicus) beef suckling cows and heifers.

Material and Methods:

Animals and location

The study was carried out in Pitangueira Group farms, located in the western border of Rio Grande do Sul, Brazil. All cows were maintained on South American natural grasslands and supplemented with mineralized salt. Heifers and cows had free access to water. For the development of the study two hundred twenty-seven (227) heifers with two years old and one hundred and forty three (143) Braford suckling cows, from 50 to 85 postpartum days were used. Body condition score (BCS) was recorded on the first treatment (Day 0) based on a 1-5 scale, 1= emaciated; 5= obese (Ayres et al., 2009).

Experimental design

Cows were placed a new and heifers once-used intravaginal device with 1.0 g of progesterone (P4, Sincrogest®, Ourofino Saude Animal, Brazil) and 2mg im injection of estradiol benzoate (EB, Sincrodiol®, Ourofino Saúde Animal, Brazil) at the beginning of the protocol (Day 0). The progesterone device was removed from cows and heifers eight days later (Day 8); subsequently, the cows were treated with 300 IU im of eCG (SincroeCG®, Ourofino Saude Animal, Brazil), 0,5 mg im of sodic cloprostenol (PGF2a; Sincrocio®, Ourofino Saude Animal, Brazil) and 1mg im estradiol cipionate (EC, SincroCP® Ourofino Saude Animal, Brazil). FTAI was proceeded 48 hours after device removal on Day 10. In addition, heifers received 0,5mg of cloprostenol on Day 0 (Fig. 1). The distribution of the follicular size on day 8 was didactic divided in seven groups (≤7, 7.1 to 8, 8.1 to 9, 9.1 to 10, 10.1 to 11, 11.1 to 12 and > 12 mm of diameter).

Ultrasound examination

All cows and heifers were submitted to transrectal ultrasound examination (6.5 MHz linear transrectal transducer; Medisono P3V, Kylumax Eletromedicina, Brazil) of the ovaries on days 0, 8 and 10 to evaluate the presence of CL and diameter of the largest follicle. Also, cows that had at least one follicle greater than 8mm on Day 8 and absence of POF on Day 10 were considered having had premature ovulation. Transrectal ultrasonography was applied to diagnose pregnancy 30 days after TAI (Day 40). The presence of viable embryonic vesicle was considered pregnant cow or heifer. P/AI was defined by dividing the number of pregnant cows 30 days after TAI by the total number of inseminated cows.

Statistical Analysis

Analysis of variance (PROC GLM; SAS Inst. Inc, Cary, NC) was used to determine the effect of follicle diameter. The means of BCS, P/AI and distribution of cows and heifers between the categories of follicles were compared by using ANOVA. Regression analysis was used to predict the effect of independent variable on dependent variable. Probabilities of less than 5% level were considered statistically significant ($P < 0.05$).

Results

The pregnancy rate per AI was similar between cows (60/143, 41.96%) and heifers (96/227, 35.56%). However, P/AI was low ($P<0.05$) in primiparous (11/51, 21.57%) compared to multiparous cows (49/92, 53.26%) and in heifers with not CL on Day 0 (13/51, 25.49%) compared to heifers with CL on day 0 (83/172, 48,26%). An effect of follicular size average per categories in cows and it impact on pregnancy rates was observed, multiparous cows (mean 9,38mm) vs. primiparous (mean 7,56mm), and between heifers with CL on Day 0 (mean 8,95mm) vs. heifers without CL (mean 7,27mm). Thus, the reduction of follicular diameter at day 8 impacts on the reduction in the pregnancy rate per AI (Fig. 2).

Pregnancy rate per AI in heifers not differ between group ≤7mm and 7.1 to 8 mm, but group ≤7mm differ ($P<0.05$) compared to the others larger than 8.1 mm, except to group > 12 mm. On cows it was the same, but group ≤7mm not differ to group > 12 mm. On the other hand, the group ≤7mm, the one of the lowest P/AI, presented around 25% of the total distribution in heifers and cows (Fig. 3).

Body condition score 3.5 at beginning of the protocol (Day 0) promoted better pregnancy rate ($P<0.05$) compared to BCS 2.5. In addition, the mean follicular diameter at Day 8 is positively correlated with BCS (Fig.4).

The follicular size mean on Day 8 for pregnant cows was 9,90mm (n=60) and nonpregnant cows 7,88mm (n=83). In pregnant heifers was 9,25mm (n=96) and nonpregnant 8,06mm (n=131). No difference between follicular size average on Day 8 between pregnant and nonpregnant cows and heifers. Heifers and cows POF size on Day 8 was around 9mm.When it divides different categories by follicles greater than or equal to 9 mm and smaller than 9 mm, POF size at Day 8 greater than or equal to 9 mm in diameter had a positive impact ($P<0.05$) on P/AI in heifers with CL on Day 0, primiparous and multiparous cows with BCS of 2.5 compared with follicle size at Day 8 smaller than 9 mm. (Table 1).

Discussion

In beef production systems, the longevity of breeding stock has a substantial effect on economic efficiency (Snelling et al., 1995). Increase retention of young cows improve production efficiency through decreased replacement rate and changing age structure of the herd resulting in a greater proportion of cows at maximal production potential for calf body weight (Roberts et al., 2015). On the other hand, primiparous beef cows typically experiences have prolonged periods of postpartum anestrus (Wiltbank, 1970), and have the highest incidence of reproductive failure (Roberts et al., 2015), reducing P/AI to FTAI programs compared with multiparous cows (Sá Filho et al., 2009). One reason for that fail are the primiparous cows average follicle size, that are smaller than the multiparous cows and it is correlated with the pregnancy rate, as show in Figure 2.

Studies performed in 1990s demonstrated that estradiol in association with progestogens are able to synchronize the ovulation and allow cows to be timed AI (TAI) (Bó et al., 1994, 1995). Although the results are now more consistent than 17 years ago, but the mean P/AI has remained around 50% (Pfeifer 2015). We suggest that distribution of the dominant follicular size at Day 8 is one of the most important reasons to keep the mean P/AI around 50%. As showed in Figure 3 around 25% both cows and heifers had follicle size in group ≤ 7mm and the P/AI in this category of follicles size are 23% (n=52) for heifers and 6% (n=34) for cows. On the other hand, observing P/AI close to 70% in heifers with dominant follicle size between 11.1 to 12 mm and 65% in cows between 10.1 to 11 mm, but just around 10% of heifers and cows had dominant follicle size between 11.1 to 12 and 10.1 to 11 mm respectively.

It is know that for production of a viable embryo requires ovulation of a competent oocyte, adequate progesterone production by the CL, and an adequate uterine environment (Perry et al., 2005). Vasconcelos et al. (2001) reported that cows induced to ovulate small follicles developed smaller CLs and secreted less progesterone compared to cows induced to ovulate larger follicles. This supporting the importance of the dominant follicle size to obtain higher levels of pregnancy in FTAI programs and to improve the reproductive efficiency of the farms.

In the present study, the BCS 2.5 led to a pregnancy rate lower than BCS 3.5 in cows and heifers. This findings are supported by Sá Filho et al. (2010) which reported that BCS at the day to insert the progesterone-releasing device of the estradiol and progesterone based FTAI programs influenced P/AI, and by Wiltbank et al. (2002), Bó et al. (2007) and

Meneghetti et al. (2009) who observed that the cows in low body condition reduced the reproductive responses to oestrus synchronization treatments. Furthermore, in this research when the BCS increase, the average of the follicular size on Day 8 increases too. Thus, high BCS somehow provide an increase in the diameter of the dominant follicle in estradiol and progesterone FTAI protocols and consequently a higher pregnancy rate compared to cows and heifers with low BCS.

Size of the POF are influenced by the number of follicular waves during the estrous cycle with larger follicles being produced by cows having two follicular waves as opposed to three (Ginther et al., 1989; Celik et al., 2005). Some studies indicated that larger follicle size being associated with reduced fertility due to aging of the oocyte (Mihm et al., 1996; Revah and Butler, 1996). At the present study we observed a decreased of P/AI for less than 30% in heifers with follicle size at Day 8 on group more than 12 mm. Similar results has been reported by Perry et al. (2007) in beef heifers that ovulated a follicle with a more extreme diameter decrease the P/AI compared to heifers that ovulated follicles >10.7 mm and <15.7 mm in diameter at time of AI. On the other hand, in this study has not observed changes to follicles size group more than 12 mm in cows on P/AI that kept around 50%. The reason for the difference between heifers and cows in P/AI when follicles were equal or more than 12 mm on Day 8 is not known. However, Byerley et al. (1987) suggested that the puberty and the number of time that heifers were in estrus before to AI effect the maturational changes associated with cycling activity. As know that cows with a larger preovulatory follicle size develop a larger diameter corpus luteum and produce more progesterone, favoring embryonic development (Ribeiro et al., 2016; Vasconcelos et al., 2001). For this reason, the pregnancy rate was divided in Table 1 by the diameter of the dominant follicles on Day 8 to less than 9 mm and greater or equal to 9 mm, a high P/AI was observed in heifers with CL on Day 0, primiparous cows and multiparous cows with BCS to 2.5 with follicles greater than or equal to 9 mm.

The present study support the hypothesis that the diameter of dominant follicle on Day 8 has effect on pregnancy rate per AI on estradiol and progesterone based FTAI. We suggest that an ultrasound analyses on Day 8 of the protocol to measure the dominant follicle size for select heifers and cows to proceed on the FTAI program and improve the PR/AI.

Acknowledgments

Authors are grateful to Carlos Eduardo Porciuncula Leonardi (PhD student - University of Saskatchewan) for his help with the statistical analyses and preparation of the manuscript and Fabrício Amadori Machado, Diego Kasali and Leandro Pacheco for collect and process the datas. The authors thank the staff of Pitangueira Group farms (Itaqui, RS, Brazil) for allowing the use their animals and facilities for this study. This research was supported by Ouro Fino Saúde Animal, Cravinhos, SP, Brazil and Pitangueiras Group farm.

Table and Figures legends

Table 1. **Pregnancy rate per AI and categories comparing animals that had follicle size greater or equal to 9 mm to smaller than 9 mm**. The analyses to check the difference to was proceed comparing the P/AI to follicle ≥ 9 mm with follicle <9 mm per category not between different categories. Letters represent the difference ($p<0.05$) between follicle ≥ 9 mm with follicle <9 mm per category. Four heifers were not recorded for the presence of CL on Day 0.
*Low animal number was not possible run the statistical analysis.

Figure 1. **Experimental design for cows and heifers.** Transretal ultrasonography (US) was proceed to measure on Day 0 the presence of CL on Days 8 & 10 the dominant follicle diameter and on Day 40 the pregnancy check (30 days after AI). On Day 0 was placed the intravaginal progesterone-releasing hormone device and injected estradiol benzoate (EB). The device was withdrawal on Day 8 and injected in the same day estradiol cipionate (EC), prostaglandin (PGF2α) and equine chorionic gonadotropin (eCG). AI was proceed 48 hours after device removal on Day 10. In addition, a dose of prostaglandin was injected in heifers

Figure 2: **Pregnancy rate per AI and follicle size average per ies (multiparous cows, primiparous cows, heifers with CL on Day 0 and heifers with no CL on Day 0)**. Letters show the difference ($P<0.05$) for P/AI between categories.

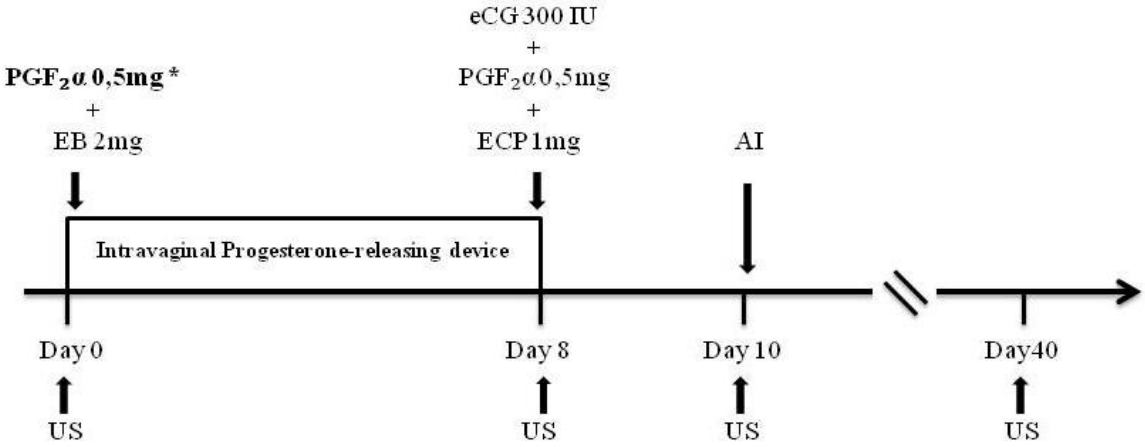
Figure 3. **Distribution of follicle size on Day 8 and respectively P/I per group (≤7, 7.1 to 8, 8.1 to 9, 9.1 to 10, 10.1 to 11, 11.1 to 12 and > 12 mm of diameter)**. The distribution was divided in heifers (A) and Cows (B). Letters represent the difference for the P/AI per group.

Figure 4. **Pregnancy rate per body condition score (BCS) on Day 0 and follicle size average for each BCS group (2.5, 3.0 and 3.5)**. Uppercase letters represent the difference ($P<0.05$) for pregnancy rate for each BCS group and lowercase letters the difference ($P<0.05$) between the follicle size average for each BCS group.

Table 1.

Categories	Follicle ≥ 9 mm		Fol < 9 mm	
	No.	*P/AI %*	*No.*	*P/AI %*
Heifers	136	[a]49.26	91	[b]31.87
Heifer with CL on Day 0	114	[a]52.63	58	[b]39.66
Heifer with No CL on Day 0	21	[a]33.33	30	[a]20.00
Primiparous	19	[a]42.11	32	[b]9.38
Multiparous	62	[a]59.68	30	[a]40.00
Multiparous ECC 2.5	33	[a]57.58	16	[b]31.25
Multiparous ECC 3.0	22	[a]59.09	13	[a]53.85
Multiparous ECC 3.5	7	[*]71.43	1	[*]0.00

Figure 1.



US: Transretal ultrassonography
- Day 0: Presence of CL
- Day 8 & 10: Dominant Follicle diameter
- Day 40: Pregnancy check (30 days after AI)

EB= Estradiol Benzoate
ECP= Estradiol Cipionate
eCG= equine Chorinonic Gonadotropin

*PGF on Day o was apply just in heifers.

Figure 2.

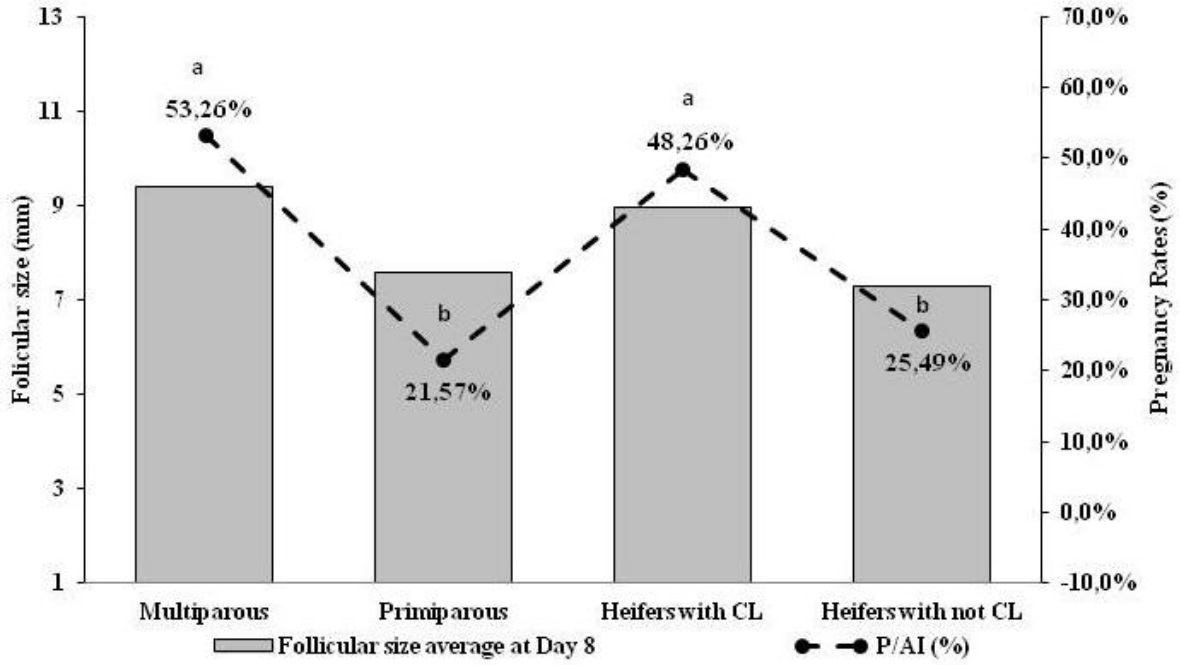

13
11
9
7
5
3
1
Follicular size (mm)
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
-10,0%
Pregnancy Rates (%)
a
53,26%
b
21,57%
a
48,26%
b
25,49%
Multiparous
Primiparous
Heifers with CL
Heifers with not CL
Follicular size average at Day 8
P/AI (%)

Figure 3.

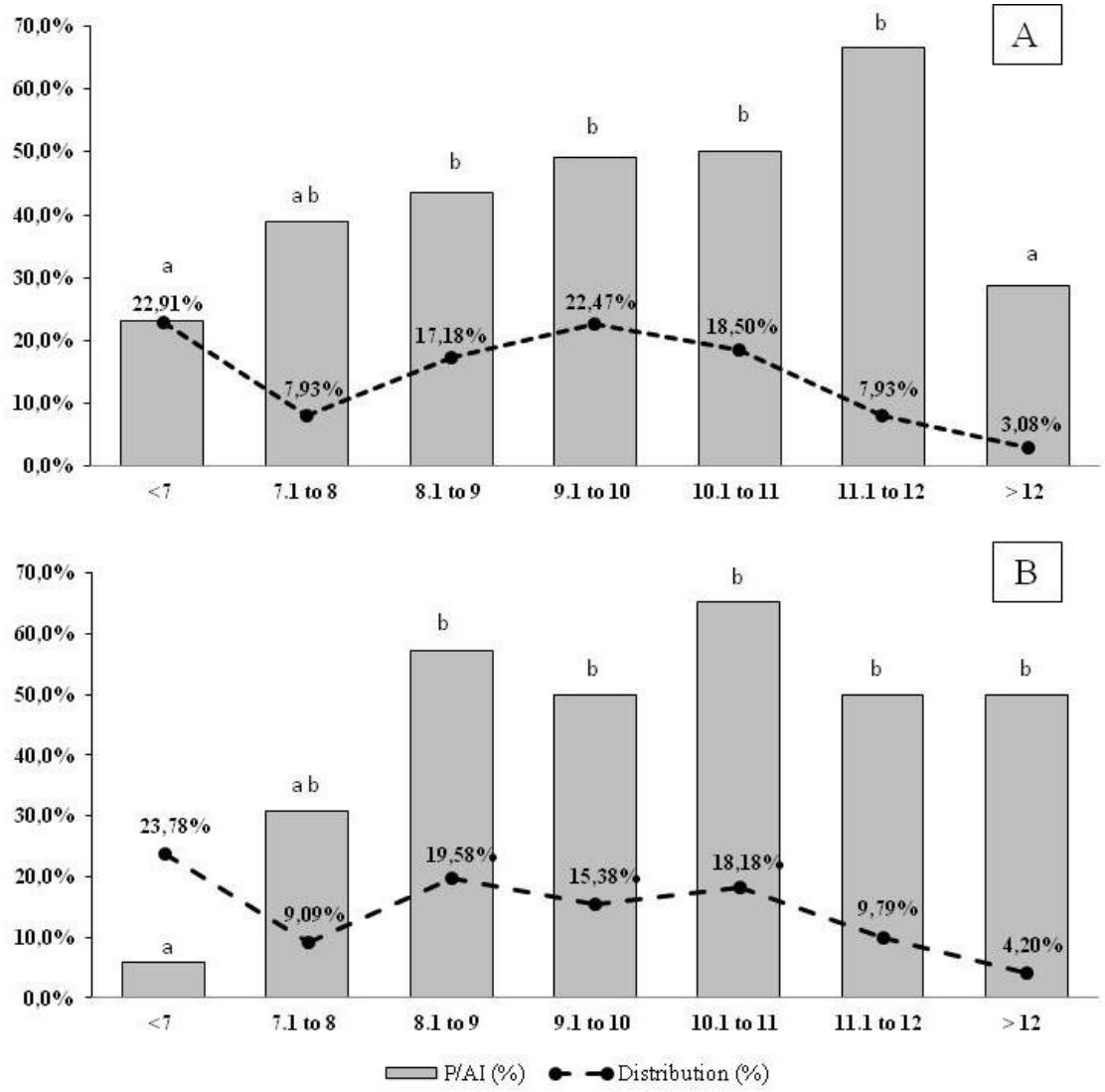
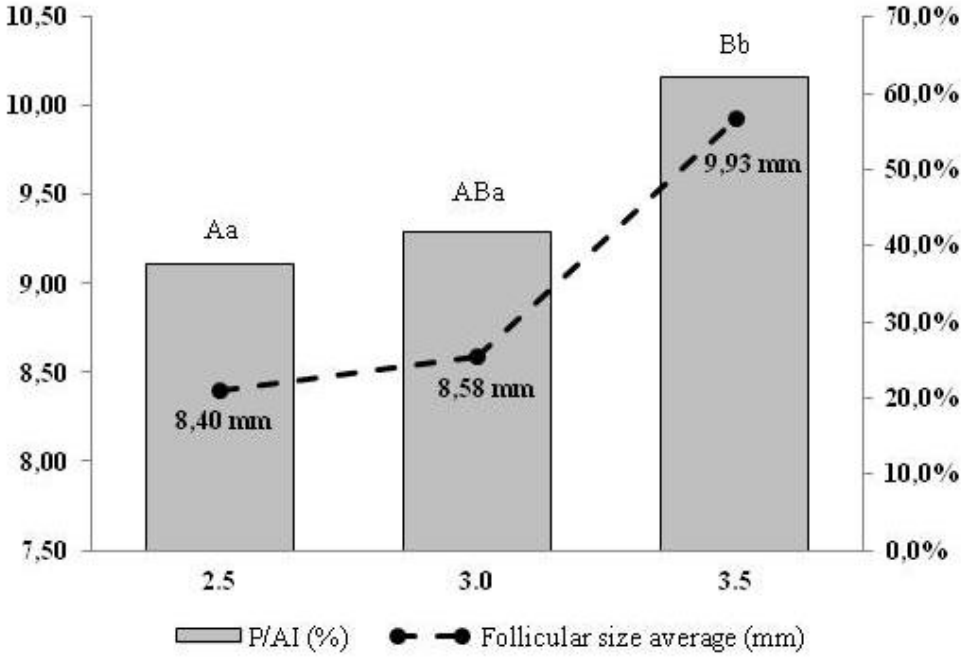

A
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
a
a b
b
b
b
b
a
22,91%
7,93%
17,18%
22,47%
18,50%
7,93%
3,08%
<7
7.1 to 8
8.1 to 9
9.1 to 10
10.1 to 11
11.1 to 12
>12
B
a
a b
b
b
b
b
b
23,78%
9,09%
19,58%
15,38%
18,18%
9,79%
4,20%
P/AI (%)
Distribution (%)

Figure 4.


10,50
10,00
9,50
9,00
8,50
8,00
7,50
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
Aa
ABa
Bb
8,40 mm
8,58 mm
9,93 mm
2.5
3.0
3.5
P/AI (%)
Follicular size average (mm)

4 CONCLUSÃO

O diâmetro do folículo dominante no momento da retirada do dispositivo liberador de progesterona em protocolos de IATF a base de estrógeno e progesterona tem influência sobre a taxa de prenhez por inseminação artificial. O presente estudo revela que o exame de ultrassonografia é uma alternativa para decisões individuais conforme o diâmetro do folículo dominante na retirada do dispositivo de progesterona em protocolos de IATF. Assim, proporciona melhor eficiência reprodutiva em função da capacidade do folículo pré-ovulatório em responder ao hormônio luteinizante e ovular o mais próximo da inseminação artificial.

5 REFERÊNCIAS

AYRES, H; FERREIRA, RM; TORRES-JÚNIOR, JRS; DEMÉTRIO, CGB; DE LIMA, CG; BARUSELLI, PS. Validation of body condition score as a predictor of subcutaneous fat in Nellore (Bos indicus) cows. **Livestock Sci**, 123: 175-179. 2009.

BARUSELLI, PS. IATF supera dez milhões de procedimentos e amplia o mercado de trabalho. **Revista CFMV**, 69: 57-60. 2016.

BARUSELLI, PS; FERREIRA, RM; SÁ FILHO, MF; BÓ, GA. Review: Using artificial insemination v. natural service in beef herds. **Animal**, 12: 45-52. 2018.

BARUSELLI; PS; FERREIRA; RM; COLLI; MHA; ELLIFF; FM. Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. **Anim Reprod**; 14: 558-571. 2017.

BINELLI, M; SUBRAMANIAM, P; DIAZ, T; JOHNSON, GA; HANSEN, TR; BADINGA, L; THATCHER, WW. Bovine interferon-τ stimulates the janus kinase-signal transducer and activator of transcription pathway in bovine endometrial epithelial cells. **Biol Reprod**, 64: 654-665. 2001.

BÓ, GA; ADAMS, GP; PIERSON, RA; MAPLETOFT, RJ. Exogenouscontrol of follicular wave emergence in cattle. **Theriogenology**, 43: 31-40. 1995.

BÓ, GA; ADAMS, GP; PIERSON, RA; TRÍBULO, H; CACCIA, M; MAPLETOFT, RJ. Follicular wave dynamics after estradiol-17 treatment of heifers with or without a progestogen implant. **Theriogenology**, 41: 1555-1569. 1994.

BÓ, GA; CUTAIA, L; PERES, LC; PINCINATO, D; MARANA. D; BARUSELLI PS. Technologies for fixed-time artificial insemination and their influence on reproductive performance of Bos indicus cattle. **Soc Reprod Fert Suppl**, 64: 223-236. 2007.

BÓ, GA; BARUSELLI, PS; MAPLETOFT, RJ. Synchronization techniques to increase the utilization of artificial insemination in beef and dairy cattle. **Anim Reprod**, 10: 137-142. 2013.

BÓ, GA; MATA, JJ; BARUSELLI, PS; MENCHACA, A. Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. **Theriogenology**, 86: 388-396. 2016.

BYERLEY, DJ; STAIGMILLER, RB; BERARDINELLI, JG; SHORT, RE. Pregnancy rates of beef heifers bred either on puberal or third estrus. **J Anim Sci**, 65: 645-650. 1987.

CELIK, HÁ; AYDM, I; SENDAG, S; DINC, DA. Number of follicular waves and their effect on pregnancy rate in the cow. **Reprod Domest Anim**, 40: 87-92. 2005.

COLAZO, MG; SEFCHEK, M; ILLUMINATI, H; MEGLIA, G; SCHMIDT, E; BÓ, GA. Fixed-time artificial insemination in beef cattle using CIDR-B devices, progesterone and estradiol benzoate. **Theriogenology**, 51: 404. 1999. Resumo.

DADARWAL, D; MAPLETOFT, RJ; ADAMS, GP; PFEIFER, LF; CREELMAN, C; SINGH, J. Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination. **Theriogenology**, 79: 859-866. 2013.

FRICKE, PM. Scanning the future--ultrasonography as a reproductive management tool for dairy cattle. **J Dairy Sci**, 85: 1918-1926. 2002.

GINTHER, OJ; KNOPF, L; KASTELIC, JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. **J Reprod Fertil**, 87: 223-230. 1989.

HANSEN, PJ. Current and future assisted reproductive technologies for mammalian farm animals. **Adv Exp Med Biol**, 752: 1-22. 2014.

KASTELIC, JP; MCCARTNEY, DH; OLSON, WO; BARTH, AD; GARCIA, A; MAPLETOFT, RJ. Estrus synchronization in cattle using estradiol, melengestrol acetate and PGF. **Theriogenology**, 46: 1295-1304. 1996.

LAMB, GC & MERCADANTE, VRG. Synchronization and artificial insemination strategies in beef cattle. **Vet Clin North Am Food Anim Pract**, 32: 335-334. 2016.

LARSON, JE; LAMB, GC; STEVENSON, JS; JOHNSON, SK; DAY, ML; GEARY, TW; KESLER, DJ; DEJARNETTE, JM; SCHRICK, FN; DICOSTANZO, A; ARSENEAU, JD. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone; prostaglandin F2alpha; and progesterone. **J Anim Sci**; 84: 332-342. 2006.

LIMA, FS; DE VRIES, A; RISCO, CA; SANTOS, JEP; THATCHER, WW. Economic comparison of natural service and timed artificial insemination breeding programs in dairy cattle. **J Dairy Sci**, 93: 4404-4413. 2010.

LUCY, MC; BILLINGS, HJ; BUTLER, WR; EHNIS, LR; FIELDS, MJ; KESLER, DJ; KINDER, JE; MATTOS, RC; SHORT, RE; THATCHER, WW; WETTEMANN, RP; YELICH, JV; HAFS, HD. Efficacy of an intravaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. **J Anim Sci**, 79: 982-995. 2001.

MACMILLAN, KL & BURKE, CR. Effects of oestrus cycle control on reproductive efficiency. **Anim Reprod Sci**, 42: 307-320. 1996.

MARTINEZ, MF; KASTELIC, JP; ADAMS, GP; MAPLETOFT, RJ. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. **Theriogenology**, 57: 1049-1059. 2002.

MENEGHETTI, M; SÁ FILHO, OG; PERES, RF; LAMB, GC; VASCONCELOS, JL. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: basis for development of protocols. **Theriogenology**, 72: 179-189. 2009.

MIHM, M; DISKIN, MG; ROCHE, JF. Regulation of follicle wave growth in cattle. **Reprod Dom Anim**, 31: 531-538. 1996.

ODDE, KG. A review of synchronization of estrus in postpartum cattle. **J Anim Sci**, 68: 817-830. 1990.

PERRY, GA; SMITH, MF; LUCY, MC; GREEN, JA; PARKS, TE; MACNEIL, MD; ROBERTS, AJ; GEARY, TW. Relationship between follicle size at insemination and pregnancy success. **Proc Natl Acad Sci U S A**, 102: 5268-5273. 2005.

PERRY, GA; SMITH, MF; ROBERTS, AJ; MCNEIL, MD; GEARY, TW. Relationship between size of the ovulatory follicle and pregnancy sucess in beef heifer. **J Anim Sci**, 85: 684-689. 2007.

PERRY, GA; SWANSON, OL; LARIMORE, EL; PERRY, BL; DJIRA, GD; CUSHMAN, RA. Relationship of follicle size and concentrations of estradiol among cows exhibiting or not exhibiting estrus during a fixed-time AI protocol. **Domest Anim Endocrinol**, 24: 15-20. 2014.

PFEIFER LFM;CASTRO NA; MELO VTO; NEVES PMA; CESTARO JP; SCHNEIDER A. Timed artificial insemination in blocks: A new alternative to improve fertility in lactating beef cows. **Anim Reprod Sci**, 163: 89-96. 2015.

RACEWICZ, P; SICKINGER, M; WŁODAREK, J; JAŚKOWSKI, JM. Ultrasonographic diagnosis of early pregnancy in cattle using different ultrasound systems. **Tierarztl Prax Ausg G Grosstiere Nutztiere**, 44: 151-156. 2016.

REVAH, I e BUTLER, WR. Prolonged dominance of follicles and reduced viability of bovine oocyte. **J Reprod Fertil**, 106: 39-47. 1996.

RIBEIRO, ES; GRECO, LF; BISINOTTO, RS; LIMA, FS; THATCHER, WW; SANTOS, JE. Biology of preimplantation conceptus at the onset of elongation in dairy cows. **Biol Reprod**, 94: 1-18. 2016.

ROBERTS, AJ; PETERSEN, MK; FUNSTON, RN. BEEF SPECIES SYMPOSIUM: Can we build the cowherd by increasing longevity of females? **J Anim Sci**, 93: 4235-4243. 2015.

RODGERS, JC; BIRD, SL; LARSON, JE; DILORENZO, N; DAHLEN, CR; DICOSTANZO, A; LAM, GC. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. **J Anim Sci**; 10: 1297-1308. 2015.

SÁ FILHO, OG; MENEGHETTI, M; PERES, RF; LAMB, GC; VASCONCELOS, JL. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows II: Strategies and factors affecting fertility. **Theriogenology**, 72: 210-218. 2009.

SÁ FILHO, MF; TORRES-JÚNIOR, JRS; PENTEADO, L; GIMENES, LU; FERREIRA, RM; AYRES, H; CASTRO E PAULA, LA; SALES, JNS; BARUSELLI, PS. Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (Bos indicus) heifers. **Anim Reprod Sci**, 118: 182-187. 2010.

SARTORI, R; FRICKE, PM; FERREIRA, JC; GINTHER, OJ; WILTBANK, MC. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. **Biol Reprod**, 65: 1403-1309. 2001.

SILVA, MAV; SANTOS, CS; FRANÇA, IG; PEREIRA, HG; SÁ FILHO, MF; FREITAS, BG; GUERREIRO, BM; FAQUIM, A; BARUSELLI, PS; TORRES-JÚNIOR, JRS. Hormonal strategy to reduce suckled beef cow handling for timed artificial insemination with sex-sorted semen. **Theriogenology**, 114: 159-164. 2018.

SNELLING, WM; GOLDEN, BL; BOURDON, RM. Within-herd genetic analyses of stayability of beef females. **J Anim Sci**, 73: 993-1001. 1995.

SPROTT; LR. Management and financial considerations affecting the decision to synchronize estrus in beef females. **J Anim Sci**; 77: 1-10. 1999.

THIBIER, M e WAGNER, HG. World statistics for artificial insemination in cattle. **Livestock Prod Sci**, 74: 2003-212. 2002.

VASCONCELOS, JL; SARTORI, R; OLIVEIRA, HN; GUENTHER, JG; WILTBANK, MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. **Theriogenology**, 56: 307-314. 2001.

VISHWANATH; R. Artificial insemination: the state of the art. **Theriogenology**, 59: 571-584. 2003.

WILTBANK, JN. Research needs in beef cattle reproduction. **J Anim Sci**, 31: 755-762. 1970.

WILTBANK, JN; GTIMEN, A; SARTORI, R. Physiological classification of anovulatory conditions in cattle. **Theriogenology**, 57: 21-52. 2002.