

# Ultrasonographic evaluation of the reproductive tract as predictors of pregnancy in Girolando heifers submitted to Timed Artificial Insemination

Avaliação ultrassonográfica do trato reprodutivo como preditor de prenhez de novilhas Girolando submetidas à Inseminação Artificial em Tempo Fixo.

Lara Nogueira Silenciato<sup>1</sup> , Joaquim Esquerdo Ferreira<sup>2</sup> , Samuel Rodrigues Bonamichi do Couto<sup>3</sup> , Otávia Reis e Silva<sup>3</sup> , Osvaldo Almeida Resende<sup>4</sup> , Andressa Ferreira da Silva<sup>5</sup> , Helcimar Barbosa Palhano<sup>6</sup> , Marco Roberto Bourg de Mello<sup>7\*</sup> 

<sup>1</sup>Veterinary, MSc. Programa de Pós-graduação em Zootecnia – PPGZ, Universidade Federal do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>2</sup>Veterinary, PhD. Centro de Ensino Superior de Valença – CESVA, Valença, RJ, Brasil

<sup>3</sup>Veterinary, MSc. Programa de Pós-graduação em Medicina Veterinária – PPGMV, Universidade Federal do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>4</sup>Veterinary, MSc. Embrapa Agrobiologia, Seropédica, RJ, Brasil

<sup>5</sup>Veterinary, PhD. Departamento de Medicina e Cirurgia Veterinária, Instituto de Medicina Veterinária – IV, Universidade Federal do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>6</sup>Veterinary, PhD. Departamento de Anatomia Animal e Humana, Instituto de Ciências Biológicas e da Saúde – ICBS, Universidade Federal do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>7</sup>Veterinary, PhD. Departamento Reprodução e Avaliação Animal, Instituto de Zootecnia – IZ, Universidade Federal do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

## Abstract

The objective of this study was to evaluate the influence of the degree of uterine and ovarian development on the pregnancy rate of Girolando heifers submitted to Timed Artificial Insemination (TAI). 56 heifers, representing 100% of the category in the herd studied, were evaluated. Prior to hormonal treatment, the heifers were submitted to a gynecological evaluation employing ultrasonography, in order to measure the diameter of the uterine horns and ovaries, as well as to evaluate the presence of corpora lutea (CL) and antral follicles. Based on the finding, all animals were classified into three scores and subsequently submitted to TAI. There was no statistical difference ( $P > 0.05$ ) in pregnancy rates between the different uterine horn and ovarian scores, as well as in relation to ovarian structures. However, a statistical difference ( $P < 0.05$ ) was observed in the percentage of animals in anestrous within ovarian scores one, two and three (83.3%, 37.5%, and 42.8%, respectively). Concluding, uterine and ovarian development did not interfere with the pregnancy rate of Girolando heifers in the herd studied.

**Keywords:** fertility, heifer selection, reproductive tract score.

## Resumo

O objetivo deste trabalho foi avaliar a influência do grau de desenvolvimento uterino e ovariano na taxa de prenhez de novilhas Girolando submetidas à Inseminação Artificial em Tempo Fixo (IATF). Foram avaliadas 56 novilhas, representando 100% da categoria no rebanho estudado. Essas novilhas foram submetidas à avaliação ginecológica prévia ao início do protocolo, com o auxílio da ultrassonografia, mensurando-se o diâmetro dos cornos uterinos e dos ovários, assim como a presença ou não de corpos lúteos (CL) e de folículos antrais. De acordo com os valores encontrados para os diâmetros uterino e ovariano, os animais foram classificados em escores de 1 a 3. Após a avaliação ginecológica, todos os animais foram submetidos a um mesmo protocolo de sincronização da ovulação, sendo inseminadas em tempo fixo. Não foi observada diferença estatística ( $P > 0,05$ ) na taxa de prenhez, entre os diferentes escores uterinos e ovarianos, assim como em relação às estruturas ovarianas. No entanto, foi observada diferença estatística ( $P < 0,05$ ) na porcentagem de animais em anestro dentro dos escores ovarianos 1, 2 e 3 (respectivamente, 83,3%, 37,5% e 42,8%). Conclui-se que o desenvolvimento uterino e ovariano não interferiu na taxa de prenhez de novilhas Girolando no contexto da população do rebanho estudado.

**Palavras-chave:** fertilidade, seleção de novilha, escore de trato reprodutivo.



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### \*Correspondence

Marco Roberto Bourg de Mello  
Departamento de Reprodução e Avaliação Animal – DRAA, Instituto de Zootecnia – IZ, Universidade Federal Rural do Rio de Janeiro – UFRRJ  
BR 465, Km 07  
CEP 23897-000 – Seropédica (RJ), Brasil  
E-mail: mmello@ufrjr.br

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## Introduction

General evaluations of the female reproductive tract, including the diameter and tone of the uterine horns and the ovarian structures (corpus luteum and antral follicles), have been used as predictors of fertility in beef (Andersen et al., 1991; Gutierrez et al., 2014) and dairy heifers (Stevenson et al., 2008).

Young et al. (2017) developed a system to evaluate the reproductive tract of dairy cows, using scores according to the size (small, medium and large) and position (pelvic or abdominal) of the uterine horns. The authors observed that females carrying smaller horns and positioned fully in the pelvic cavity are more fertile (higher conception rate) than those with larger horns, and already positioned within the abdominal cavity.

Male and female fertility is a factor that refers to the various components and stages required to consider them functionally capable to go through all phases of the reproductive cycle, from the fertilization of the ovum to the birth of the offspring (Foote, 2003).

In general, characteristics that are indicative of fertility require systematic evaluation and a good RMS (Reproduction Management System) to avoid any financial losses by helping the technician responsible making correct decisions while carrying out the reproduction work in herds (Patterson et al., 2000; Cammack et al., 2009; Parker Gaddis et al., 2017).

In relation to heifer fertility, reduced age at first calving (about 22 months) has generally been seen to negatively affect the first lactation, however, on the other hand, milk production during the life of the animal and overall profitability of the dairy herd is increased (Zavadilová & Stipková, 2013).

There are artificial ways to introduce heifers into the reproductive life early on, and one of them is the use of Timed Artificial Insemination (TAI), which is biotechnology based on hormone therapy for the synchronization and induction of ovulation. This method allows to optimize the breeding management by shortening time interval of the insemination period, while making estrous detection unnecessary, providing two key advantages. Thus, TAI is the best way to introduce accurate genetics in a herd through Artificial Insemination (Macedo et al., 2015).

The objective of the present study was to evaluate, with the aid of ultrasonography, the influence of uterine and ovarian development on the pregnancy rate of Girolando heifers submitted to TAI.

## Material and methods

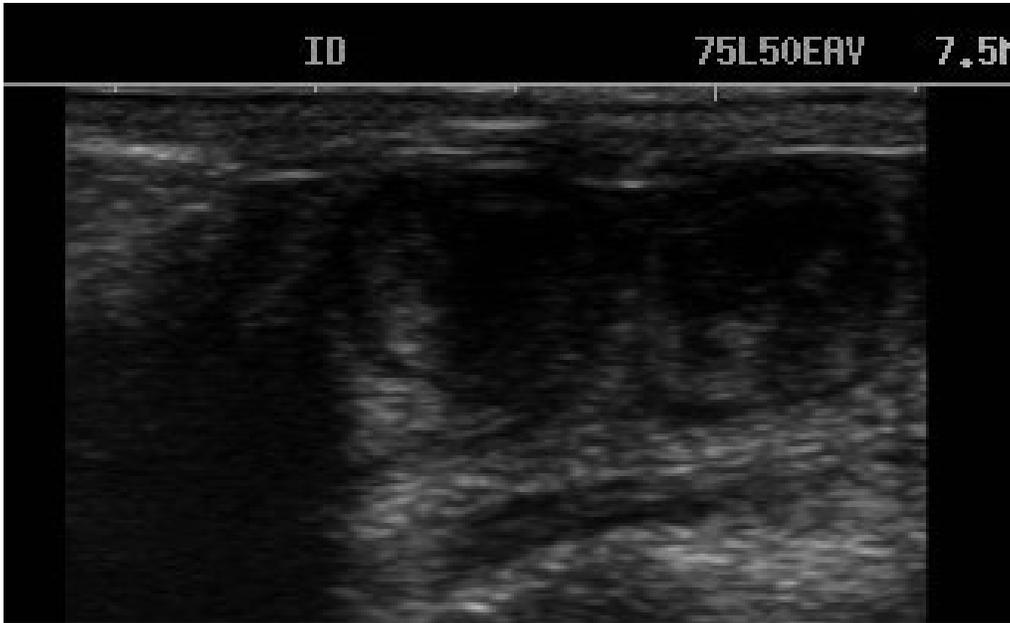
The present study was approved by the Ethics Committee on the Use of Animals of the Universidade Federal Rural do Rio de Janeiro (23083.002854 / 2012-03).

This experiment was carried out in the State of Rio de Janeiro by the Agricultural Research Company (PESAGRO-RIO) in the area of Dairy Cattle of the State Center of Research in Organic Agriculture (CEPAO) located in Seropédica-RJ (latitude: 22°46'56. S, longitude: 43°39'41. W), from August 2014 to February 2016.

The PESAGRO-RIO herd consisted of 255 Girolando animals (belonging to different genetic groups from ½ to ¾ Holstein-Gir), including 162 cows, (primiparous and multiparous), 56 heifers and 37 calves. The study used fifty-six heifers, who had a mean age of 26 months and a mean weight of 370 kg. All the heifers of the herd were used in this study, representing 21.96% of the total population. The animals were tuberculosis-brucellosis-free with a body condition score (BCS) of 2.5 (Houghton et al., 1990). They were maintained on an extensive system with *Brachiaria* pasture (*Brachiaria decumbens*) and access to water and mineral salt *ad libitum*.

Prior to executing the protocol of synchronized ovulation, a gynecological evaluation on all animals was performed by rectal palpation and ultrasound examination (Mindray DP 2200-Vet, 7.5MHz transrectal linear transducer (Sao Paulo/Brazil). After clinical examination, none of the evaluated females presented congenital or acquired infections of the genital tract (vaginitis, follicular cyst, tortuous cervix, and total or unilateral ovarian hypoplasia) that could potentially interfering with pregnancy after TAI.

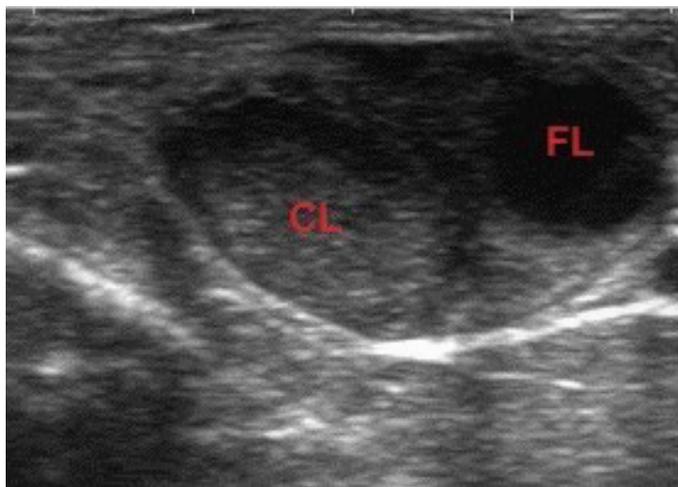
The diameters of the right and left horns were measured using ultrasonography, after the transverse cut at the bifurcation (Figure 1), when the two uterine horns could be observed separately, as described by Holm et al. (2016). Three uterine horn measurements were taken from



**Figure 1.** Ultrasound cross-sectional image of uterine horns at bifurcation.

each individual, subsequently, based on the calculated mean, classified into scores from 1 to 3, as following: score 1:  $\emptyset < 1.4\text{cm}$ ; score 2:  $1.4 \leq \emptyset < 1.7\text{cm}$ ; score 3:  $\emptyset \geq 1.7\text{cm}$ .

The ovaries were evaluated in relation to their diameter and the presence of corpus luteum (CL), as well as antral follicles (Figure 2). The follicles were assessed according to their diameter and afterwards classified as larger or smaller than 8mm (Ginther, 2016). Ovaries that presented only follicles less than 8mm, the female was categorized as "Anestrous type I", and follicles larger than 8mm, as "Anestrous type II", according to Peter et al. (2009) and Ginther (2016). In this way, animals that had a CL at the time of evaluation were classified as cyclic and those with only follicles (regardless of diameter) were classified as acyclic. The value used for the classification of the ovary score was calculated by the mean of the two largest diameters of the right and left ovary. With the ultrasonographic data from the ovarian diameter, the females were classified into scores from 1 to 3, as following: 1:  $\emptyset < 2\text{ cm}$ ; score 2:  $2 \leq \emptyset < 2.4\text{cm}$ ; score 3:  $\emptyset \geq 2.4\text{cm}$ .



**Figure 2.** Ultrasound image of the cyclic heifer ovary with the presence of a corpus luteum (CL) and an antral follicle (FL).

In order to perform the Timed Artificial Insemination, the nulliparous females, on a random day of the estrous cycle, considered day 0 (DO), received an intravaginal device containing 1g of progesterone (P4), (DIB®, MSD Saúde Animal, Brazil), 2mg of estradiol benzoate (RIC-BE®, Tecnopec, Brazil) and 500µg of cloprostenol intramuscularly (Ciosin®, MSD Animal Health, Brazil). On D8, 500µg of cloprostenol (Ciosin®, MSD Saúde Animal, Brazil) was intramuscularly injected and the progesterone device was removed. On D10, 48h after removal of the device, 50 micrograms of Buserelin Acetate (Sincroforte®, Ouro Fino, Brazil) was applied intramuscularly, following a modified protocol regarding the time of insemination used by Silva et al. (2008). The diagnosis of pregnancy was performed by ultrasound 30 days after insemination. The pregnancy rate (number of pregnant animals/number of inseminated animals) was calculated and was then associated with the ovarian and uterine scores.

Statistical analysis was performed using the chi-square test and Fisher exact test when necessary, with a significance level of 5% (p <0.05) to compare the pregnancy rates in relation to the uterine and ovarian scores, the presence of ovarian structures and the relation between ovarian score and cyclicity using GraphPad Prism v5.0® for windows®.

## Results and discussion

The relationship between the uterine score and pregnancy rate is presented in Table 1 and shows the total number of animals, the uterine evaluation score and the respective pregnancy rates of Girolando heifers submitted to TAI. There was no statistical difference (p=0.1487; X<sup>2</sup>=3.811, 2) between the pregnancy rates of the three uterine scores.

The results presented in Table 1 suggest that, in the studied population, the uterine development did not affect the pregnancy rate in a TAI protocol under the conditions evaluated. These results can be explained by the age, weight and body condition that these animals presented at the beginning of the TAI protocol. The heifers of the present study had a mean age of 26 months, mean weight of 370kg and a body condition score between 2.5 and 3.5 (range from 1 to 5).

**Table 1.** Effect of uterine and ovarian score on the pregnancy rate of Girolando heifers submitted to Timed Artificial Insemination.

Scores	Pregnancy rate / Uterine Score (%)	Pregnancy rate / Ovarian Score (%)
1	63.6 <sup>a</sup>	61.1 <sup>a</sup>
2	64.0 <sup>a</sup>	75.0 <sup>a</sup>
3	78.0 <sup>a</sup>	57.0 <sup>a</sup>

- The values with different superscripts within the same column differ significantly (p < 0.05). - Uterus Diameter - Score 1: Ø <1.4cm; Score 2: 1.4 ≤ Ø <1.7cm; Score 3: Ø ≥ 1.7cm. - Ovary Diameter Score 1: Ø <2 cm; Score 2: 2 ≤ Ø <2.4cm; Score 3: Ø ≥ 2.4cm.

These physical conditions of the heifers could mean that they have already reached sexual maturity, and thus, the uterine development is complete, implying in an absence of interference of this variable on pregnancy rate. This fact was confirmed by Holm et al. (2016) who, when evaluating genital development in heifers using ultrasonography, concluded that the development of the reproductive system is influenced both by age and live weight, and animals that have reached sexual maturity are not influenced anymore by the uterine diameter on conception or the pregnancy rate.

However, the results found in the present experiment disagree with those found by Holm et al. (2009) when evaluating 272 heifers, through rectal palpation and ultrasonography, with an average of 14 months old. They concluded that the evaluation of the reproductive tract score (combined assessment of diameter and uterine tone) is a good predictor of fertility.

In another study, Holm et al. (2015) analyzed data from a Bovelder herd (South African crossbred) composed of 12-15-month-old heifers. Although the age is different from the present study, the authors also concluded that an evaluation of the reproductive tract score is a valid management tool to improve reproductive efficiency over the long term, excluding heifers that probably would not breed or could be born late during their first breeding season, and therefore detrimental to the reproductive performance of the subsequent season. This difference of results is possibly explained by the use of different breeds, as well as the different ages of the animals.

The ovarian scores and their relationship to the pregnancy rates are presented in Table 1. No statistical difference ( $p > 0.05$ ) was observed between the three ovarian scores and pregnancy rates, thus, the variable "ovarian size" did not influence the pregnancy rate in the present study.

Ireland et al. (2011) stated that fertility is linked to the presence of antral follicles and that the follicular reserve is not affected by the stage of the estrous cycle. These same authors also concluded that ovary size is associated with body development and also with the presence of a corpus luteum. Therefore, it is inferred that the size of the ovary is not directly related to the number of antral follicles, but rather to the stage of the estrous cycle in which the female is found.

Also, in terms of ovarian development, the results of the present study are in agreement with the findings by Holm et al. (2016) who did observe that the ovary length variables were independently associated with reproductive outcomes in this study (pregnancy failure). These authors also point out that one of the possible explanations for not finding a difference between ovary size and pregnancy failure may be due to a measurement error in ovary length at the time of data collection, thus making this analysis difficult. However, they observed that the size of the ovary could be a reflex of antral follicles count, size or presence of follicle and/or corpus luteum. This last statement, based on the findings of Holm et al. (2015), is also in agreement with the other data obtained in the present experiment.

Table 2 shows the percentage of cyclic Girolando heifers (presence of CL) and acyclic heifers (absence of CL independently of the size of the largest follicle present) at the beginning of the TAI protocol according to the ovarian score. The group of animals presenting an ovarian score 1 had a higher number of acyclic females ( $p < 0.0001$ ,  $X^2 = 50.41$ , 2) when compared to the other groups (ovarian scores 2 and 3). These data corroborate with the findings of Nascimento et al. (2003) who observed an influence of ovary size varying according to the pubertal state of the animal (pre-pubertal < peri-pubertal < puberty), reproductive stage and estrous cycle phase. Thus, acyclic animals have a smaller ovarian size.

**Table 2.** Percentage of cyclic and acyclic heifers at the beginning of the Timed Artificial Insemination protocol according to the ovarian score.

Ovarian scores	Animals	Cyclic (%)	Acyclic (%)
1	18	16.7 <sup>a</sup>	83.3 <sup>b</sup>
2	24	62.5 <sup>a</sup>	37.5 <sup>a</sup>
3	14	57.2 <sup>a</sup>	42.8 <sup>a</sup>

- The values with different superscripts within the same column differ significantly ( $p < 0.05$ ). - Ovary Diameter - Score 1:  $\emptyset < 2$  cm; Score 2:  $2 \leq \emptyset < 2.4$  cm; Score 3:  $\emptyset \geq 2.4$  cm.

Table 3 presents the pregnancy rate of Girolando heifers submitted to a TAI program in relation to the presence or absence of ovarian structures at the beginning of the protocol. No statistical difference ( $p > 0.05$ ) was observed between the groups.

**Table 3.** Effect of stratification of the ovarian condition on the pregnancy rate of Girolando heifers submitted to Timed Artificial Insemination.

Ovarian structures	Cyclicity	Pregnancy rate (%)
Corpus Luteum	Cyclic	69.2 <sup>a</sup>
Anestrous Type I	Acyclic	50.0 <sup>a</sup>
Anestrous Type II**	Acyclic	66.7 <sup>a</sup>

Results presented in Table 3 are inconsistent with those found by Kasimanickam et al. (2016), who observed that the physiological state of the ovarian structures (presence of CL) of the animal at the beginning of the TAI protocol affects the pregnancy rate, that is, cyclic pubertal heifers (CL presence) had better results than the pre-pubertal (acyclic).

Furthermore, results in Table 3 disagree with the findings of Gutierrez et al. (2014) who reported a lower pregnancy rate in animals that did not present ovarian structures compared to animals

with corpus luteum or follicles larger than 10 mm. In addition, the results found by LeFever & Odde (1986) *apud* Rosenkrans & Hardin (2003), who concluded that the pubertal state in which the animal is entering the reproductive season influences the pregnancy rate, that is, animals that have initiated their reproductive season cycling, have greater chance to become pregnant. This difference can be justified by the different methodologies used to evaluate the follicles (8mm vs 10mm).

## Conclusion

The uterine and ovarian development did not interfere in the pregnancy rate of Girolando heifers in the context of the studied population and under the conditions of the present experiment. Thus, ultrasound evaluation of the uterine and ovarian diameter of heifers under these conditions was not able to provide a better selection to improve the pregnancy rate. However, an earlier evaluation of the gonadal development of Girolando heifers using ultrasonography, which was not object of the present study, may constitute an important gynecological evaluation tool to guide the beginning of the reproductive management of this category.

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