

## ARTIFICIAL INSEMINATION IN DEEP INTRACORNIAL FIXED TIME IN BOVINE

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**Abstract**– The search for improvements in the efficiency of the artificial insemination (AI) technique is still required. The objective of this study was to verify if intracornial fixed-time artificial insemination can improve conception rates concerning transcervical IATF (Artificial Insemination in fixed time). The experiment was developed with 68 cows, submitted to the following protocol: D0 at 08:00 h, and received an intravaginal P4 release device associated with intramuscular (IM) Estradiol Benzoate. D8 at 08:00 h, the P4 device was withdrawn and Dinoprost tromethamine (IM) was administered; Estradiol Cypionate (IM) and Equine Chorionic Gonadotrophin (IM). D10 at 08:00 h, 34 cows were inseminated in the uterine body and 34 inseminated intracornial, ipsilateral to the ovary with the preovulatory follicle. The diagnosis of gestation was performed 45 days after AI by transrectal ultrasonography. The conception rate found with semen deposition in the uterine body was 32%, while deep intracornial deposition was 26%. The results demonstrated a non-significant difference between the techniques ( $p > 0.05$ ). Thus, uncontractual fixed-time artificial insemination does not improve the conception rate when compared to artificial insemination at fixed transcervical times. However, more studies need to be done to standardize the technique.

### INTRODUCTION

The National production system needs the constant development of programs of easy application and high reproductive and productive efficiency for dairy and beef cattle (Baruselli, 2016). Considering the frank expansion of the milk and beef market, it is necessary to increase the productivity and genetic quality of the herds, to meet the growth prospects of the consumer market, as well as to improve reproductive control by reducing production costs (Grundemann, 2016). In this context, the genetic improvement of the herd is a decisive action to increase the productivity (Grillo, 2016).

Insemination Artificially (IA) is one of the tools

available, capable of promoting genetic improvement, increasing reproductive efficiency and productivity of cattle herds (Firmino *et al.*, 2021; Inforzatto *et al.*, 2008; Junior *et al.*, 2021; Morais *et al.*, 2020; Silva *et al.*, 2021).

However, AI has some limitations, such as the need for estrus observation, an alternative to optimizing such limitations is the use of ovulation synchronization, performed through Artificially insemination in Fixed Time (IATF) (GRILLO, 2016). Bó *et al.* (2003), consists of using some of the various existing hormonal protocols developed to control follicular growth and induce ovulation, without the need to detect estrus.

Senger *et al.* (1988), artificially cornial

insemination offer advantages when compared to insemination in the uterine body, and also highlighted the positive aspect of technical training for correct semen deposition. Meirelles *et al.* (2012) achieved significant results between the two techniques. As per Assis Junior (2018), there was no statistically significant difference between the groups.

The advantage over the deposition of semen in the uterine body compared to the deposition in the cervix does not generate any more doubts (Meirelles *et al.*, 2012). However, doubts are still raised regarding the best place for semen deposition after the cervix (López-Gatius, 2000).

Thus, the research aims to compare the conception rates in cut cows inseminated by IATF-IC and IATF-TC, evaluating whether artificial intracornual fixed-time insemination can improve conception rates concerning transcervical.

## MATERIALS AND METHODS

Approved by the Ethics Committee on the Use of Animals, under the process of n° 23101.1002958/2018-30.

Carried out in the farm field of the municipality of Conceição of the Araguaia, southeastern Pará, with latitude: 08°15'28"; longitude: 49°15'54" (IBGE, 2010), being conducted between September and November, 2018.

We used 68 multiparous Nellore cows, a mean age of 48 months, body condition score (ECC) of around 2.5, considering the scale from 1 to 5 (1 very thin and 5 very fat), with at least 45 days postpartum.

All females were reared in the extensive system of *brachiaria* grass pickets (*Braquiariabrizanta*) with access to water and mineral salt *ad libitum*.

Cows that did not present, at the time of evaluation, reproductive tract abnormalities, and no history of abortion were considered to participate in the experiment.

All cows were evaluated by ultrasound (Honda® - HS1500V, multifrequency rectal transducer using frequency 7.5 MHz), to confirm or not pregnancy, then the non-pregnant females were submitted to the synchronization protocol.

The protocol used was three passages; on the First day, considered (D0), to 08:00 h, the animals received an intravaginal progesterone release device (P4) (DIB® 1.0 g) associated with 2.0 mg of Estradiol Benzoate (2.0 mL of GONADIOL® 1 mg/ml) via

intramuscular (IM). On the 8th (D8), To 08:00 h, the P4 device was withdrawn and 12.5mg of Dinoprost tromethamine was administered (2.5 ml of Lutalyse® with 5 mg/ml) (IM), 1.0 mg Estradiol Cypionate (0.5 mL E.C.P.® with 2 mg/ml) (IM) and 300IU of Gonadotrophine Corionic Equine (1.5 ml of NOVORMON® with 200 IU/ml) (IM). On day 10 (D10), at 08:00 h artificially inseminations were performed.

Females submitted to estrus synchronization protocol and ovulation induction were randomly divided into two groups for artificially insemination at fixed times (IATF), Group 1: IATF-Transcervical (n=34) using the Universal Applicator and Group 2: IATF-Intra Cornual (n=34) using the inovulator for embryo transfer. Before insemination, group 2 cows were examined by ultrasound to identify the dominant follicle and its respective ovary.

All managements performed with the groups were performed on the same day. Group 1 was the first to be inseminated and properly identified with a fire mark in the termite region, and group 2 was also inseminated.

To inseminate the females, Aberdeen Angus bull semen was used, stored in a 0.25 reed mL and 35 million sperm per dose, acquired from an accredited commercial semen processing plant, presenting after thawing, turbilhonamento 3, motility 40%, and vigor 3.

After insemination, all cows were kept separate, without contact with the bull or with other cows that were not part of the experiment, for 45 days, under identical environment and nutrition conditions as already described. Then, the diagnosis of pregnancy with an ultrasound device was made (Honda® - HS1500V, multifrequency rectal transducer using frequency 7.5 MHz).

Semen analysis, protocol, inseminations, and pregnancy diagnosis were performed by the same technician.

Data were collected in the field and later tabulated in a spreadsheet in Microsoft Excel. The statistical analysis was performed consisting of simple and crossed frequency tables and the chi-square test.

Statistics were considered significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

The total conception rate was 29% (20/68) of Nellore cows. The animals of the group 1 (IATF/TC), who

were submitted to artificially insemination technique in Fixed Transcervical Time using conventional device presented a conception rate of 32% (11/34), while group 2 animals (IATF-IC) that were submitted to intracornual Fixed Time Artificial Insemination technique in which the device conventionally used for embryo transfer was used, the rate of 26% (09/34) was obtained. There was no statistically significant difference between the groups (P-value = 0.5945) (Table 1).

The design rates obtained for the group IATF-TC 32% (11/34) and the group IATF-IC 26% (09/34) were smaller than those obtained by Assis Junior (2018), who obtained a pregnancy rate of 52% for the intracornual technique, while for the transcervical technique it resulted in 47%. Our results were also lower than those found by Momont *et al.* (1989), with insemination cows in the ipsilateral horn 55% and contralateral 48% to the ovulatory follicle.

The three studies showed no statistically significant difference between the groups studied, and the highest rates found by Assis Junior (2018) e Momont *et al.* (1989) be attributed to the fact that they inseminate cows that presented follicles above 11mm in D10 and have performed insemination using natural heat respectively, while in this study all animals were inseminated regardless of follicle size and without using natural heat.

The presence of an FD greater than 11mm at the time of insemination is an indicator of better ovarian response and the conception rate of bovine females submitted to IATF (Ribeiro Filho *et al.*, 2013).

Dalton *et al.* (1999) state that deep intracornual artificially insemination favors sperm access to the oocyte, but does not guarantee better pregnancy rates. But Senger *et al.* (1988), state that cornual artificially insemination offers advantages when related to insemination in the uterine body, considering the rates they found of 64.6% and 44.7% respectively for the techniques mentioned, also highlighted the positive aspect of training the technician for correct semen deposition.

Meirelles *et al.* (2012) also achieved significant results between the two techniques, 48.8% in the body of the uterus and 67.4% intracornual. Thus, contrary to the results found in this search. Senger *et al.* (1988) conducted the training insemen for six months and the two studies performed insemination in the natural heat of the cows.

The training carried out in this study for deposition of the semen of the uterine horn lasted one month. In bovine females, training to perform insemination is for transcervical semen deposition (Assis Junior, 2018). The time spent per cow to inseminate the IATF-TC group was up to two minutes, while the time spent per cow to inseminate the IATF-IC group was above two minutes.

The time required to perform the AI act in bovine females interferes with the conception rate, when carried out in a period of up to two minutes better conception rates are achieved (Vendruscolo *et al.*, 2005). In our study, the IATF-CI, the time spent to perform it was higher than IATF-CT, which may also be an explanation for lower conception rates obtained.

Nevertheless, Verberckmoes *et al.* (2004) state that the deposition of semen in the uterine horn favors spermatozoa reducing their losses. When a greater amount of semen is deposited at the uterus-tuberos junction, it can contribute positively to forming a perforated reserve, conception rate, and pregnancy (Dalton *et al.*, 1999), second Kodithuwakku *et al.* (2007), there is evidence that sperm increases contractions of the oviduct by accelerating its transport to the site of fertilization.

Thus, the time allocated to training for semen deposition in the uterine horn may have been insufficient, thus requiring a longer period of training, which could consequently have contributed to increasing the precision in the deposition of the semen in the horn, and could reduce the time spent to deposition it (Table 2), and have influenced the result obtained in this research of not presenting the significant statistical difference

**Table 1.** Result of post-estrus synchronization and ovulation cows submitted to intracornual IATF using devices used for embryo and transcervical transfer using the conventional device.

| Group     | Full animals (n) | Full cows Pregnant (n) | Full cows not Pregnant (n) | Design rate (%) |
|-----------|------------------|------------------------|----------------------------|-----------------|
| IATF/TC*  | 34               | 11                     | 23                         | 32 <sup>a</sup> |
| IATF/IC** | 34               | 09                     | 25                         | 26 <sup>a</sup> |
| Full      | 68               | 20                     | 48                         | 29              |

\*Artificially Insemination in Transcervical Fixed Time; \*\* Intracornual Fixed Time Artificially Insemination.

between the techniques used.

According to the specialized literature Almeida *et al.* (2016); Torres (2015); Vasconcelos *et al.* (2009). In Piauí, 150 Nellore cows, multiparous, ECC between 2.5 and 3.5, were separated into two blocks and submitted to hormonal protocol, the group BEDT56, with calf at the foot, was placed with four vasectomized bulls and 56-hour weaning, while the control group (NESD) remained with calf at the foot and without the presence of bull, among the results found on the rate of pregnant 69.3% (BEDT56) and 37.3% (NESD) (Almeida *et al.*, 2016). Corroborating the conception rate and conditions found in this study, since the cows were ECC around 2.5, without biostimulation and with baby-siture.

Already Torres (2015) reports, that the ECC interferes with the probability of pregnancy rate in pregnancy IATF, the increase of 0.5 units of ECC implied a 39.0% increase in the probability of pregnancy. And the temporary removal of calves associated with the hormonal protocol used may increase pregnancy rates due to higher ovulation rates (Vasconcelos *et al.*, 2009). The association of biostimulation, use of vasectomized bulls, and temporary weaning increase follicular development, conception rate, and pregnancy in cows (Almeida *et al.*, 2016).

At Guaruja Farm in Iporã-PR, 86 Nellore cows, body score seven, kept under grazing, supplemented with protein salt and water *ad libitum*, were randomly divided into three experimental groups for transcervical insemination, Group 1 (N=35) inseminated with a single dose of commercial semen, Group 2 (N=26) inseminated twice in a row and the Group 3 (N=25) were inseminated twice, with an interval of 12 hours, the gestation rates obtained 31.43% (11/35), 34.62% (9/26) and 20.00% (5/25) respectively were similar ( $p < 0,05$ ) (Thomazi *et al.*, 2010). Conception rates were identical to that found in this research, where

**Table 2.** Average time spent to deposit the semen in each technique in the reproductive tract of Nellore cows through artificially insemination in fixed-transcervical time and artificially insemination in fixed-intraconual time.

| Technique | Time spent approximately |
|-----------|--------------------------|
| IATF-TC   | 1min e 8s                |
| IATF-IC   | 4min e 4s                |

\* Artificially Insemination in Fixed Transcervical Time;

\*\* Intracornual Fixed Time artificially Insemination

cows were inseminated with a single dose of commercial semen of spermatic concentration of 35 million, which after thawing as recommended, the semen used in the study was evaluated with 40% motility and 3 vigour.

Assis Junior (2018) used doses rated for sperm concentration of 37.5 million, motility 75%, and vigor 4, classification is above that found in the doses used in this study (35 million sperm, motility 40%, vigor 3), therefore, the quality of semen in the present study may also be one of the factors that contributed to the conception rates obtained.

The pregnancy rates with intracornual insemination found by the cited author are higher than those found in this study, which was 26%, in the cited literature it was possible to verify the controversy that exists about the results obtained since the conception rates obtained hour differ ed by time not statistically different when the AI techniques used compared IATF-IC with IATF-TC methods that take into account the place of deposition of the semen.

With the accomplishment of this study, the controversy is maintained, because in the comparison to the techniques the conception rates did not present statistical differences, suggesting the need for greater control of some factors such as: the longer training period of the technician for intracornual semen deposition; improve the selection and identification of cows to be inseminated concerning the evaluation of the size of the FD; making a good selection of cows considering the condition of body score; choice and handling of the semen used; temporary weaning during the protocol and it is also necessary to take into account the time of year taking into account the supply and scarcity of food.

### Conflict of Interest

There is no conflict of interest between the authors. all authors contributed directly to the article.

### CONCLUSION

1. In this study, intracornual fixed-time artificially insemination did not improve the conception rate when compared to transcervical fixed-time artificially insemination.
2. However, further research is needed to standardize the technique.

## REFERENCES

- Almeida, P.S., Silva Filho, M.L., Lopes, G.S., Kunkel, D., Machado Júnior, A.A.N., Rocha, H.B., Braga Aguiar, H.M.V.S. and Soares, R.S. 2016. Bioestimulação e desmame temporário, objetivando a dinâmica folicular, taxa de concepção e de prenhez em vacas Nelore submetidas a IATF. *Revista Brasileira de Reprodução Animal*. 40(4): 229-231.
- Assis Junior, V.F. 2018. Inseminação artificial intracornual com protocolos de IATF em fêmeas da raça Nelore. 42 f. Dissertação (Mestrado em Ciências Animais) – Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Brasília.
- Baruselli, P.S. 2016. IATF supera dez milhões de procedimentos e amplia o mercado de trabalho. *Revista CFMV*. 22(69): 57-60.
- Bó, G.A., Baruselli, P.S. and Martínez, M.F. 2003. Pattern and manipulation of follicular development in *Bos indicus* cattle. *Animal Reproduction Science*. 78: 307-326.
- Dalton, J.C., Nadir, S., Bame, J.H. and Saacke, R.G. 1999. Effect of a deep uterine insemination on spermatozoa accessibility to the ovum in cattle: a competitive insemination study. *Theriogenology*. 51(5): 883-890.
- Firmino, A.A.F. and Chagas, J.C.C. 2021. Inseminação Artificial em Tempo Fixo (IATF) em bovinos de corte na Fazenda Alfredo de Maya no município de Cacimbinhas/AL. *Diversitas Journal*. 6(4): 4159-4170.
- Grillo, G.F., Mello, M.R.B., Guimarães, A.L.L., Resende, O.A., Alves, P.A.M., Silva, A.F., Jesus, V.L.T. and Palhano, H.B. 2016. Taxa de prenhez de vacas leiteiras em programa de inseminação artificial em tempo fixo com e sem triagem ginecológica. *Revista Brasileira de Medicina Veterinária*. 38(2): 187-194.
- Grundemann, J.T. 2016. Desempenho reprodutivo de novilhas, vacas desmamadas e vacas amamentando submetidas ao protocolo de inseminação com detecção de estro e em tempo fixo (IAETF), seguido de ressincronização. 61 f. Dissertação (Mestrado em Ciência Animal) – Universidade Federal do Pampa, Rio Grande do Sul.
- IBGE. Instituto Brasileiro de Geografia e Estatística. Conceição do Araguaia. 2010. Available in: <<https://cidades.ibge.gov.br/brasil/pa/conceicao-do-araguaia/panorama>>. Access in: 25 may. 2022.
- Inforzato, G.R., Santos, W.R.M., Climeni, B.S.O., Dellalibera, F.L. and Filadelpho, A.L. 2008. Emprego de IATF (Inseminação Artificial em Tempo Fixo) como alternativa na reprodução da pecuária de corte. *Revista Científica Eletônica de Medicina Veterinária*. 6(11).
- Junior, R.A.B. and Cardoso, A.R. 2021. Inseminação artificial em tempo fixo (iatf): revisão bibliográfica. *Arquivos Brasileiros de Medicina Veterinária FAG*. 4(2): 218-225.
- Kodithuwakku, S.P., Miyamoto, A. and Wijayagunawardane, M.P. 2007. Spermatozoa stimulate prostaglandin synthesis and secretion in bovine oviductal epithelial cells. *Reproduction*. 133: 1087-1094.
- López-Gatius, F. 2000. Site of semen deposition in cattle: a review. *Theriogenology*. 53(7): 1407-1414.
- Meirelles, C., Kozicki, L.E., Weiss, R.R., Segui, M.S., Souza, A., Santos, I.W. and Breda, J.C.S. 2012. Comparison between deep intra cornual artificial insemination (dIAI) and conventional artificial insemination (AI) using low concentration of spermatozoa in beef cattle. *Brazilian Archives of Biology and Technology*. 55(3): 371-374.
- Momont, H.W., Seguin, B.E., Singh, G., Stasiukynas, E. 1989. Does intrauterine site of insemination in cattle really matter? *Theriogenology*. 32(1): 19-26.
- Morais, S.B., Orlandi, C.M.B., Gasparotto, P.H.G., Dantas Filho, J.V., Cavali, J., Júnior, L.D.C. and Daudt, C. 2020. Custos e benefícios da inseminação artificial em pequenas propriedades leiteiras. *Agrarian*. 13(48): 249-264.
- Ribeiro Filho, A.L., Ferraz, P.A., Rodrigues, A.S., Bittencourt, T.C.B.S.C., Loiola, M.V.G. and Chalhoub, M. 2013. Diâmetro do folículo no momento da inseminação artificial em tempo fixo e taxa de concepção em vacas Nelore. *Ciência Animal Brasileira*. 14(4): 501-507.
- Senger, P.L., Becker, W.C., Davidge, S.T., Hillers, J.K. and Reeves, J.J. 1988. Influence of cornual insemination on conception in dairy cattle. *Journal of Animal Science*. 66(11): 3010-3016.
- Silva, M.A.N., Mello, M.R.B. and Palhano, H.B. 2021. Inseminação artificial e inseminação artificial em tempo fixo em bovinos. *Revista Científica do UBM*. 79-97.
- Thomazi, S., Pinto Neto, A., Mota, M.F., Silva, R.Z. and Martínez, A.C. 2010. Taxa de gestação de vacas nelore inseminadas artificialmente em tempo fixo com diferente concentração espermática e momento de inseminação. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR*. 13(2): 105-108.
- Torres, H.A.L., Tineo, J.S.A. and Raidan, F.S.S. 2015. Influência do escore de condição corporal na probabilidade de prenhez em bovinos de corte. *Arquivos Zootecnia*. 64(247): 255-260.
- Vasconcelos, J.L.M., Vilela, E.R. and Sá Filho, O.G. 2009. Remoção temporária de bezerros em dois momentos do protocolo de sincronização da ovulação GnRH-PGF2 $\alpha$ -BE em vacas Nelore pós-parto. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 61(1): 95-103.
- Vendruscolo, M., Kozicki, L.E., Segui, M.S., Weiss, R.R. and Santos, I.V. 2005. Relação entre a duração do ato da inseminação artificial em bovinos e a fertilidade. *Archives of Veterinary Science*. 10(2): 81-88.
- Verberckmoes, S., Soom, A.V., Pauw, I., Dewulf, J., Vervaeck, C. and Kruif, A. 2004. Assessment of a new uterotubal junction insemination device in dairy cattle. *Theriogenology*. 61(1): 103-115.