PRELIMINARY NOTE

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Impact of sperm hyperactivation and estrus on pregnancy in timed AI Bos indicus beef cows*

Impacto da hiperativação espermática e do estro na prenhez em vacas de corte Bos indicus inseminadas em tempo-fixo

Luiz Francisco Machado Pfeifer[1]; George Moreira da Silva[2]; Vanessa Lemos de Souza[2]; Jair Sábio Oliveira Júnior[3]; Jéssica de Souza Andrade[3]; José Roberto Potiens[4]

[1] Empresa Brasileira de Pesquisa Agropecuária, Porto Velho – RO, Brazil
[2] Universidade Federal de Rondônia, Programa de Pós-graduação em Desenvolvimento Regional e Meio Ambiente, Porto Velho – RO, Brazil
[3] Universidade Federal de Rondônia, Programa de Pós-graduação em Biodiversidade e Biotecnologia, Porto Velho, RO – Brazil
[4] Seleon Biotecnologia, Itatinga – SP, Brazil

ABSTRACT

This study hypothesized that cows that express estrus within 48 hr of progesterone insert removal ovulate earlier, and that the association between estrus and the degree of sperm hyperactivation increases pregnancy rates in beef cows. In Experiment 1, Nelore cows were divided into two groups according to the expression of estrus within 48 hr after progesterone removal: Estrus (E48, n = 19) and No Estrus (NE48, n = 13). Cows from the E48 group ovulated earlier (P < 0.02; 73.3 ± 13.1 h vs. 95.5 ± 37.5 h). In Experiment 2, 480 postpartum beef cows were treated as described in Experiment 1. The semen from the two bulls used for timed artificial insemination was classified as Hyper-activated (H+) and Non-Hyper-activated (H-). The batches of H+ and H- semen of each bull used to inseminate cows were distributed in E48 and NE48 cows. The pregnancy per AI of cows from the E48H+ (60.7%, 91/150) and E48H- (65.7%, 88/134) groups was greater (P < 0.02) compared with cows from NE48H+ (51.6%, 66/128) and NE48H- (41.2%, 28/68) groups. Cows that exhibited estrus ovulated earlier and had higher pregnancy rates. The kinematics of the semen used in this study did not influence the fertility of cows, regardless of estrus incidence.

Keywords: Estrus. Sperm hyper-activation. Ovulation.

RESUMO

Este estudo levantou a hipótese de que vacas que expressam estro dentro de 48 horas após a retirada do dispositivo de progesterona ovulam mais cedo, e que a associação entre o estro e o grau de hiperativação espermática aumenta a taxa de prenhez de vacas de corte *Bos indicus*. No Experimento 1, vacas Nelore foram separadas em 2 grupos de acordo com a ocorrência de estro em até 48h após a retirada do dispositivo de progesterona: estro (E48, n = 19) e sem estro (SE48, n = 13). As vacas do grupo E48 ovularam antecipadamente (73,3 ± 13,1 *versus* 95,5 ± 37,5 h; P < 0,02). No Experimento 2, 480 vacas Nelore pós-parto foram tratadas como no Experimento 1. O sêmen de 2 touros foi classificado em hiperativado (H+) e não hiperativado (H-). Partidas H+ e H- de ambos os touros foram utilizadas para inseminar vacas classificadas como E48 e SE48. A prenhez por IA das vacas dos grupos E48H+ (60,7%, 91/150) e E48H- (65,7%, 88/134) foi maior (P < 0,02) em comparação às vacas dos grupos SE48H+ (51,6%, 66/128) e SE48H- (41,2%, 28/68). Vacas em cio ovularam mais cedo e tiveram maior taxa de prenhez e a cinética do sêmen utilizado nesse estudo não afetou a prenhez das vacas independente da incidência do estro.

Palavras-chave: Estro. Hiperativação espermática. Ovulação.

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Correspondence to:
Luiz Francisco Machado Pfeifer
Empresa Brasileira de Pesquisa Agropecuária
Rodovia BR-364, Km 5,5, Zona Rural
CEP: 76815-800, Porto Velho – RO, Brazil
e-mail: luiz.pfeifer@embrapa.br

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The integration of technological advancements in beef operations holds the potential to profoundly enhance the productivity and sustainability of farms situated within the Amazon Biome (Freitas Junior & Barros, 2021). In this scenario, the use of timed artificial insemination (TAI) in beef cows can improve the reproductive and economic efficiency of meat production systems (Baruselli et al., 2012; Bó & Baruselli, 2014). Although TAI is a well-established technique, the proportion of inseminated cows in Pará and Rondônia, large meat-producing states in the Amazon, is below the Brazilian average of 23% (Associação Brasileira de Inseminação Artificial, 2022). Increased pregnancies per artificial insemination (P/AI) are expected to encourage broader adoption of TAI programs among livestock producers. In that regard, Pfeifer et al. (2019) observed that the association between female ovarian response and sperm kinematics increases P/AI in postpartum cows. Despite its significant findings, the authors' methodology has limitations. Specifically, it requires the involvement of a qualified professional and the use of ultrasound equipment to assess ovarian follicle size in each female at the time of TAI. Non-invasive or less labor-intensive approaches could significantly advance the refinement of the TAI protocols, ultimately leading to enhanced reproductive efficiency and fertility outcomes in cattle. Given that estrus expression within 48 hr of intravaginal progesterone device (IPD) removal is often indicative of earlier ovulation and, subsequently, greater P/AI, a comprehensive investigation into this process, specifically its association with the degree of sperm hyperactivation, is highly pertinent.

Based on these considerations, the objective of this study was to evaluate the relationship between post-thaw sperm kinematics and the estrus in Bos indicus cows included in TAI protocols. This study hypothesized that cows that exhibit estrus within 48 hr of IPD removal ovulate earlier, and that the association between estrus and the degree of sperm hyperactivation increases the fertility of Nelore cows raised in the Amazon Biome.

All experimental procedures presented in this manuscript were approved by Embrapa's Committee for Ethics in Animal Experimentation (Protocol 04/2017). Experiment 1 was conducted at the Embrapa Rondônia Experimental Farm. Primiparous Nelore cows (n = 32; *Bos indicus*) with body condition scores (BCS) between 2.75 - 3.5 (on a scale of 1-5, where 1 = emaciated and 5 = obese; (Ayres et al., 2009)) were used. The cows were kept in *Brachiaria brizantha* grazing systems with free access to water and mineral salts.

All animals received an IPD (1.9 g, CIDR®, Pfizer Animal Health, São Paulo, Brazil) and estradiol benzoate at a dose of 2 mg administered intramuscularly (IM; Gonadiol®, Zoetis, São Paulo, Brazil) on day 0 of the TAI protocol. After the removal of the intravaginal device on day 8, the cows were administered equine chorionic gonadotropin (300 IU/IM; eCG, Novormon™, Syntex, Buenos Aires, Argentina), d-cloprostenol, a PGF2α analogue (150 µg/IM; Croniben®, Biogénesis-Bagó, Curitiba, Brazil), and estradiol cypionate (1 mg/IM; ECP, E.C.P®, Pfizer Animal Health, São Paulo, Brazil). At the time of IPD removal, all cows were marked in the sacrocaudal region using a marking stick to identify the animals that exhibited estrus within 48 hr of IPD removal. Animals with >75% of the ink removed were considered to be in estrus 48 hr after IPD removal. They were divided into two groups based on estrus detection up to 48 hr after IPD removal: Estrus (E48, n = 19) and No Estrus (NE48, n = 13).

The ultrasonographic evaluation (SIUI CTS-900, Guangdong, China) for follicular diameter measurement and ovulation confirmation was performed as described by Pfeifer et al. (2019).

In Experiment 2, 480 Nelore cows (*Bos indicus*), between 45 and 100 days postpartum, multiparous, and with a BCS of 2.5 to 3.5, were used. The animals were obtained from a commercial farm located in the municipality of Candeias do Jamari (RO, Brazil) and kept on *Brachiaria brizantha* pasture with free access to water and mineral salts.

The TAI protocol and estrus detection were performed as described in Experiment 1. TAI was performed 48 hr after IPD removal. Cows in estrus were inseminated before those not in estrus.

The bull semen used in this study was chosen from a semen stock that had previously been subjected to computer-assisted sperm analysis (CASA; Ivos-Ultimate™; Beverly, MA, USA) to characterize the sperm kinematics of each batch (adapted from Pfeifer et al. 2019). The following variables

were used to characterize the semen samples as hyper-activated (H+) or non-hyper-activated (H-): amplitude of lateral head displacement (ALH), linearity (LIN), and curvilinear velocity (VCL). Semen batches considered H+ exhibited ALH >6.5 µm, LIN <51%, and VCL >145 µm/s. Semen batches considered as H- exhibited: ALH ≤6.5 µm, LIN ≥51% and VCL ≤145 µm/s (Table 1). Two bulls that exhibited H+ and H- batches were used for TAI in this study.

The H+ and H- semen samples were used to inseminate cows that expressed estrus (E48) and cows that did not exhibit estrus (NE48) within 48 hr after the removal of the IPD. Therefore, the animals were distributed into the following groups: E48H+ (n = 150), E48H- (n = 134), NE48H+ (n = 128), and NE48H- (n = 68).

Transrectal ultrasound exams were performed at the time of TAI and 30 days later to measure the preovulatory follicles (POF) and detect pregnancy, respectively.

For single-point outcome variables (POF diameter, ovulation time, and follicular growth rate), the effects of treatment, parity, and BCS were included in the model. Parity and BCS did not have significant effects and were consequently excluded from the model. Therefore, these variables were analyzed by ANOVA, and Tukey's test was used to determine differences among groups. In Experiment 1, the ovulation rate was analyzed using the chi-squared test. In Experiment 2, pregnancy per AI was analyzed using logistic regression in the GLIMMIX procedure. The initial model considered group, days postpartum, BCS, estrus, degree of hyper-activation, and bull. Days postpartum, BCS, and bull did not affect the model. Therefore, only estrus and the degree of hyperactivation were kept in the final model. The relationship between POF diameter and the probabilities of estrus expression in TAI and pregnancy was determined by the formula $y = exp\ (\alpha x + b)\ /\ \left[1 + exp\ (\alpha x + b)\right]$. In all analyses, the significance level was set at $P < 0.05$.

Data on the ovarian responses of the cows in Experiment 1 are presented in Table 2. Cows in the E48 group ovulated earlier (P = 0.02) than those in the NE48 group. There were no differences in dominant follicle diameter 48 hr after IPD removal (P = 0.11), POF diameter (P = 0.53), follicular growth rate (P = 0.32), or ovulation rate (P = 0.78) between the groups.

The data for P/AI and POF diameters according to estrus occurrence and the degree of sperm hyperactivity in Experiment 2 are presented in Table 3. Cows that exhibited estrus had a greater POF diameter (P = 0.001) and P/AI (P = 0.01) than cows that did not express estrus. No differences in POF diameter and P/AI between cows inseminated with H+ and H- semen were observed (Table 3).

Table 1 – Analysis of semen parameters, according to Computer-assisted sperm analysis (CASA), of batches of sperm with different kinematic characteristics

Bull	1	1	2	2
Semen type (batch)	H-	H+	H-	H+
ALH (µm)	5,42	6,95	5,8	6,82
LIN (%)	52,61	50,74	56,86	50,2
VCL (µm/s)	120	149	131	154

ALH: amplitude of lateral head displacement; LIN: linearity; VCL: curvilinear velocity.

Table 2 – Ovarian responses of Nelore cows that expressed estrus and did not express estrus within 48 h of implant removal

	Estrus within 48h	No estrus within 48h	P-Value
Dominant Follicle Diameter 48 h after CIDR removal (mm)	12.0 ± 0.4	10.9 ± 0.5	0.11
Diameter of the Preovulatory Follicle (mm)*	12.7 ± 0.5	13.2 ± 0.6	0.53
Growth Rate of Preovulatory Follicle (mm/day)**	1.3 ± 0.13	1.5 ± 0.15	0.32
Moment of Ovulation (hours)***	73.3 ± 6.0	95.5 ± 7.4	0.02
Ovulation Rate	94.7% (18/19)	92.3% (12/13)	0.78

*Follicle is detected and measured during the ultrasound examination before ovulation; **Between CIDR removal and ovulation; ***After CIDR removal.

Table 3 – Pregnancy rate and preovulatory follicle diameter according to estrus expression and degree of sperm hyper-activation

	Estrus Expression		Degree of Hyper-activation		P-Value		
	Estrus	No estrus	H+	H-	Estrus	Hyper-activity	Estrus*Hyper-activity
POF diameter (mm)	13.5 ± 0.2^{A}	12.1 ± 0,3^{B}	13.3 ± 0.3	13.0 ± 0.2	0.001	0.38	0.35
Pregnancy/AI	63.0%^{A} (179/284)	47.9%^{B} (94/196)	56.5% (157/278)	57.4% (116/202)	0.01	0.25	0.40

^{AB}Different letters indicate a difference between groups (P<0.05). *Estrus – Hyperactivity interaction

The proportion of pregnant cows inseminated with H+ and H- semen, according to estrus expression, is shown in Figure 1. Cows from the E48H- group had a higher ($P \leq 0.05$) P/AI than cows from the NE48H+ and NE48H- groups. There was no difference ($P > 0.05$) in the P/AI between cows from the E48H+ and NE48H+ groups. Contrarily, cows from the E48H+ group had a higher ($P \leq 0.05$) P/AI than cows from the NE48H- group (Figure 1).

The probability of a cow expressing estrus ($P = 0.01$) and pregnancy ($P = 0.04$) increased with increasing POF diameter (Figure 2).

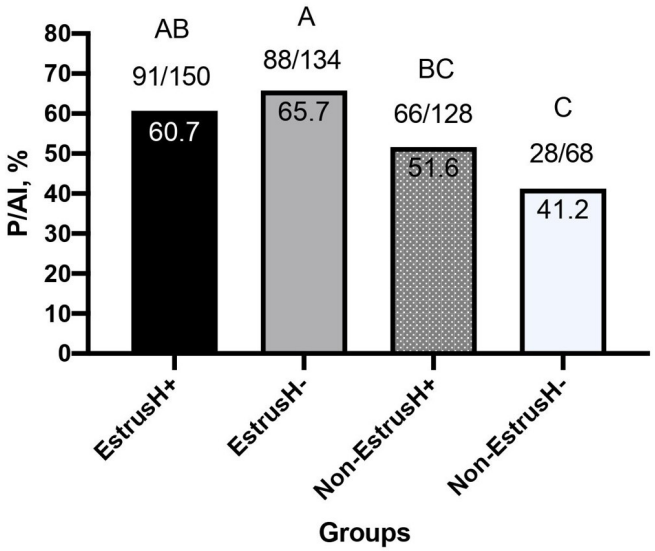

Figure 1 – Pregnancy per AI in lactating beef cows according to estrus expression at 48 hr after removal of the intravaginal progesterone device when there was insemination with sperm classified as Hyperactivated (H+) and Non-Hyperactivated (H-). Different letters indicate the effect between groups ($P < 0.05$).

These results confirmed the hypothesis that cows that exhibited estrus within 48 hr of IPD removal ovulated earlier than those that exhibited estrus after 48 hr. However, the hypothesis that the association between the degree of sperm hyperactivation and the occurrence of estrus increases pregnancy rate in cows subjected to TAI programs was not supported.

In this study, estrus identification within 48 hr of IPD removal was a good predictor of cows that ovulated earlier. Cows that did not exhibit estrus ovulated approximately 20 hr later than those that exhibited. In this regard, the higher P/AI observed in cows that exhibited estrus in Experiment 2 was associated with greater synchronization between TAI and ovulation.

In this study, the degree of sperm hyperactivation in the semen samples was determined using the mean values of ALH, LIN, and VCL. Studies observed these kinematic variables (Araya-Zúñiga et al., 2023; Dorado et al., 2013; Ibanescu et al., 2020; Marques et al., 2023; Silva et al., 2023) and the hyperactivation attributes of frozen-thawed bull semen (Schmidt & Kamp, 2004; Shojaei et al., 2012). Shojaei et al. (2012) found a significant correlation between these kinematic variables and the hyperactivation traits of frozen-thawed bull semen. In the present study, we expected that cows in estrus inseminated with H+ semen would have higher pregnancy rates than cows in estrus inseminated with H- semen. Although we observed differences in sperm kinematics between H+ and H- semen batches, these differences were not as pronounced as those observed in a previous study (Pfeifer et al. 2019; ALH 9 µm, LIN 49% and VCL 195 µm/s). Hence, concerns might arise that the kinematics of H+ and H- semen batches were not sufficiently different to result in different P/AI in

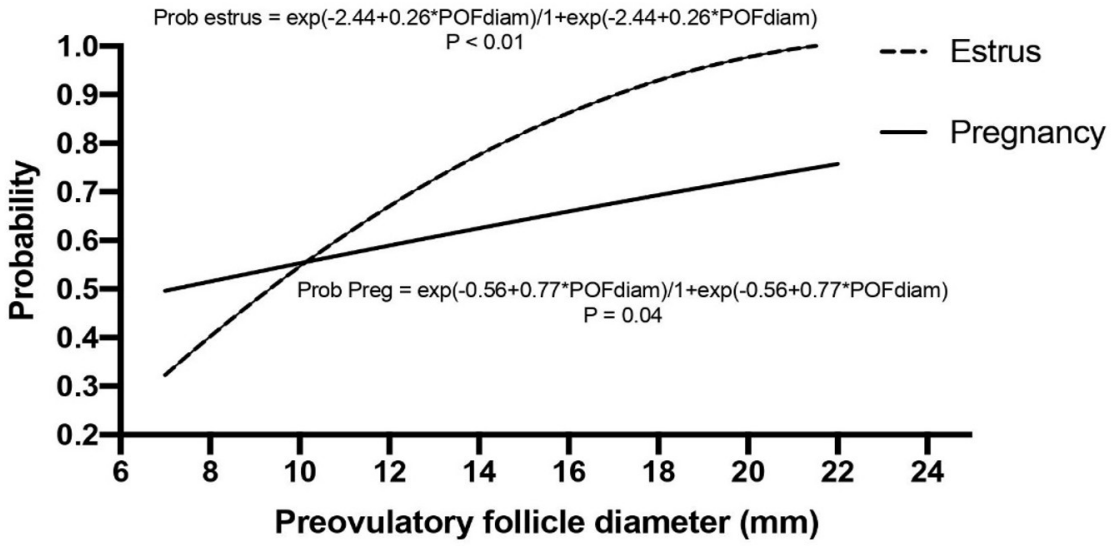

Figure 2 – Probabilities of estrus at TAI and pregnancy* in beef cows according to the diameter of the preovulatory follicle (POF) at TAI. *Pregnancy was detected 30 days after insemination.

cows subjected to TAI. Therefore, further studies involving larger batches of bulls with different kinematics are necessary to elucidate the relationship between female estrus and the degree of sperm hyperactivation during pregnancy in cows enrolled in TAI programs.

Although not different, the similar P/AI between EstrusH+ and Non-EstrusH+ groups suggests that hyperactivated semen (H+) may exert a positive effect in females expressing delayed estrus (>48 hr), which typically exhibit lower P/AI in comparison with cows that exhibit estrus (Nogueira et al., 2019; Sá Filho et al., 2010) Despite its valuable scientific contributions, a limitation of the present study is the exclusive assessment of sperm kinematics, without including additional functional tests of sperm. This aspect requires further investigation in future research.

In conclusion, cows that exhibited estrus during TAI ovulated earlier and had a greater P/AI than cows that did not exhibit estrus after IPD removal. However, the degree of sperm hyper-activation did not affect pregnancy rate in beef cows, regardless of estrus status at TAI. Additionally, the findings of this study have practical implications for improving reproductive management in beef cattle under tropical conditions. Estrus expression observed within 48 hr post-IPD removal was identified as a reliable marker for earlier ovulation. This crucial temporal relationship ensured optimal synchrony between the AI procedure and the ovulatory event, thereby improving pregnancy outcomes. Although sperm hyperactivation did not significantly influence the overall pregnancy rate, the comparable P/AI between cows in estrus and non-estrus groups inseminated with H+ semen suggests that hyperactivated semen may help mitigate the reduced fertility typically observed in cows that did not exhibit estrus. Therefore, the strategic use of semen batches with higher levels of hyperactivation may be considered a supportive tool in specific reproductive scenarios, particularly in females with delayed or absent estrus expression.

Conflict of Interest

The authors have no conflicts of interest in this study.

Ethics Statement

All experimental procedures presented in this manuscript were approved by Embrapa's Committee for Ethics in Animal Experimentation (Protocol 04/2017).

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References

Araya-Zúñiga I, Sevilla F, Molina-Montero R, Roldan ERS, Barrientos-Morales M, Silvestre MA, Valverde A. Kinematic and Morphometric Assessment of Fresh Semen, before, during and after Mating Period in Brahman Bulls. Animals (Basel). 2023;14(1):132. https://doi.org/10.3390/ani14010132. PMid:38200863.

Associação Brasileira de Inseminação Artificial. Index Associação brasileira de inseminação artificial. Uberaba: ASBIA; 2022.

Ayres H, Ferreira RM, de Souza Torres-Júnior JR, Demétrio CGB, de Lima CG, Baruselli PS. Validation of body condition score as a predictor of subcutaneous fat in Nelore (Bos indicus) cows. Livest Sci. 2009;123(2-3):175-9. https://doi.org/10.1016/j.livsci.2008.11.004.

Baruselli PS, Sales JNS, Sala RV, Vieira LM, Filho MFS. History, evolution and perspectives of timed artificial insemination programs in Brazil. Anim Reprod. 2012;9(3):139-52.

Bó GA, Baruselli PS. Synchronization of ovulation and fixed-time artificial insemination in beef cattle. Animal. 2014;8(Suppl 1):144-50. https://doi.org/10.1017/S1751731114000822. PMid:24844128.

Dorado J, Acha D, Ortiz I, Gálvez MJ, Carrasco JJ, Díaz B, Gómez-Arrones V, Calero-Carretero R, Hidalgo M. Relationship between conventional semen characteristics, sperm motility patterns and fertility of Andalusian donkeys (Equus asinus). Anim Reprod Sci. 2013;143(1-4):64-71. https://doi.org/10.1016/j.anireprosci.2013.10.003. PMid:24210876.

Freitas Junior AM, Barros PHB. A expansão da pecuária para a Amazônia legal: externalidades espaciais, acesso ao mercado de crédito e intensificação do sistema produtivo. Nova Econ. 2021;31(1):303-33. https://doi.org/10.1590/0103-6351/5064.

Ibanescu I, Siuda M, Bollwein H. Motile sperm subpopulations in bull semen using different clustering approaches – Associations with flow cytometric sperm characteristics and fertility. Anim Reprod Sci. 2020;215:106329. https://doi.org/10.1016/j.anireprosci.2020.106329. PMid:32216932.

Marques L, Missio D, de Mattos K, Cibin FW, Leivas FG, Brum DS. Correlation between in vitro sperm kinetic, oxidative stress assessments and field fertility of cryopreserved bull semen. Semina: Ciênc Agrár. 2023;44(5):1841-58. https://doi.org/10.5433/1679-0359.2023v44n5p1841.

Nogueira E, Silva MR, Silva JCB, Abreu UPG, Anache NA, Silva KC, Cardoso CJT, Sutovsky P, Rodrigues WB. Timed artificial insemination plus heat I: effect of estrus expression scores on pregnancy of cows subjected to progesterone–estradiol-based protocols. Animal. 2019;13(10):2305-12. https://doi.org/10.1017/S1751731119000442. PMid:30982488.

Pfeifer LFM, Oliveira Júnior JS, Potiens JR. Effect of sperm kinematics and size of follicle at ovulation on pregnancy rate after timed AI of beef cows. Anim Reprod Sci. 2019;201:55-62. https://doi.org/10.1016/j.anireprosci.2018.12.009. PMid:30587383.

Sá Filho MF, Crespilho AM, Santos JEP, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Anim Reprod Sci. 2010;120(1-4):23-30. https://doi.org/10.1016/j.anireprosci.2010.03.007. PMid:20395079.

Schmidt H, Kamp G. Induced hyperactivity in boar spermatozoa and its evaluation by computer-assisted sperm analysis. Reproduction. 2004;128(2):171-9. https://doi.org/10.1530/rep.1.00153. PMid:15280556.

Shojaei H, Kroetsch T, Wilde R, Blondin P, Kastelic JP, Thundathil JC. Moribund sperm in frozen-thawed semen, and sperm motion end points post-thaw and post-swim-up, are related to fertility in Holstein AI bulls. Theriogenology. 2012;77(5):940-51. https://doi.org/10.1016/j.theriogenology.2011.09.026. PMid:22137769.

Silva CS, Vianna da Costa-E-Silva E, Nunes Dode MA, Mendes Cunha AT, Garcia WR, Sampaio BFB, Borges Silva JC, Vaz FEM, Kerns K, Sutovsky P, Nogueira E. Semen quality of Nellore and Angus bulls classified by fertility indices and relations with field fertility in fixed-time artificial insemination. Theriogenology. 2023;212:148-56. https://doi.org/10.1016/j.theriogenology.2023.09.001. PMid:37722295.