PAB Pesquisa Agropecuária Brasileira 60 years

Veterinary Science/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Short IVF combined with gradient sperm selection could effectively help rescue low-viability bull semen

Abstract – The objective of this work was to evaluate different sperm selection strategies for producing in vitro fertilization (IVF) blastocysts from low-viability bull semen. Two experiments were conducted. In experiment 1, a control treatment and four semen selection techniques were compared: swim-up, glass wool, and density gradients combined with either short or long IVF. In Experiment 2, the highest-yielding selection technique was further evaluated across 10 low-viability semen samples. The protocol combining gradient selection with short IVF was the only approach that successfully yielded blastocysts. Although blastocyst rates ranged from 2 to 18%, viable embryos were still obtained from samples that would otherwise be unsuitable for embryo production. The combination of gradient selection and short IVF enables blastocyst development from low-viability semen. Despite lower success rates compared with the control, short IVF blastocysts were obtained from the majority of low-viability sperm samples.

Index terms: bulls, fertilization, resource use efficiency.

Franco Alejandro Dellavalle(✉)
Instituto Nacional de Tecnología Agropecuaria, Estacíon Experimental Agropecuaria Mercedes, Laboratorio de Biotecnología de la Reproducción, Argentina.
Email: dellavalle.franco@inta.gob.ar

Andres Rogberg-Muñoz
Universidad de Buenos Aires, Facultad de Agronomía, Dto. Producción Animal, Cidade Autônoma de Buenos Aires, Argentina.
Email: arogberg@agro.uba.ar

Juan Manuel Benitez
Instituto Nacional de Tecnología Agropecuaria, Estacíon Experimental Agropecuaria Mercedes, Laboratorio de Biotecnología de la Reproducción, Argentina.
Email: benitez.juan@inta.gob.ar

Domingo Emilio Aguilar
Instituto Nacional de Tecnología Agropecuaria, Estacíon Experimental Agropecuaria Mercedes, Laboratorio de Biotecnología de la Reproducción, Argentina.
Email: aguilar.domingo@inta.gob.ar

Rafael Fernandez-Martin
Universidad de Buenos Aires, Facultad de Agronomía, Dto. Producción Animal, Cidade Autônoma de Buenos Aires, Argentina.
Email: martinf@agro.uba.ar

Amada Eugenia Ynsaurralde-Rivolta
Instituto Nacional de Tecnología Agropecuaria, Estacíon Experimental Agropecuaria Mercedes, Laboratorio de Biotecnología de la Reproducción, Argentina.
Email: ynsaurralde.eugenia@inta.gob.ar

✉ Corresponding author

Received
October 27, 2025

Accepted
June 24, 2026

How to cite
DELLAVALLE, F.A.; ROGBERG-MUÑOZ, A.; BENITEZ, J.M.; AGUILAR, D.E.; FERNANDEZ-MARTIN, R.; YNSAURRALDE-RIVOLTA, A.E. Short IVF combined with gradient sperm selection could effectively help rescue low-viability bull semen. **Pesquisa Agropecuária Brasileira**, v.61, e04295, 2026. DOI: https://doi.org/10.1590/S1678-3921.pab2026.v61.04295.

FIV curta combinada com seleção espermática por gradiente pode resgatar sêmen de touro de baixa viabilidade

Resumo – O objetivo deste trabalho foi avaliar diferentes estratégias de seleção de esperma para a produção de blastocistos via fertilização in vitro (FIV) a partir de sêmen de touro com baixa viabilidade. Dois experimentos foram conduzidos: No experimento 1, um tratamento controle e quatro técnicas de seleção de sêmen foram testados: swim-up, lã de vidro e gradientes de densidade combinados com FIV de curta duração e longa duração. No experimento 2, o protocolo de maior rendimento foi avaliado posteriormente em 10 amostras de sêmen com baixa viabilidade. O protocolo que combina seleção de gradiente com FIV de curta duração foi a única abordagem que produziu blastocistos com sucesso. Embora as taxas de blastocistos tenham variado entre 2 e 18%, embriões de FIV ainda puderam ser obtidos a partir de amostras que, de outra forma, seriam inadequadas para a produção de embriões. A combinação da seleção por gradiente com a fertilização in vitro de curta duração permite o desenvolvimento de blastocistos a partir de espermatozoides de baixa viabilidade. Embora as taxas de sucesso tenham sido menores do que as dos grupos de controle, é possível obter blastocistos na maioria das amostras.

Palavras-chave: touros, fecundação, uso eficiente de recursos.

This is an open-access article distributed under the Creative Commons Attribution 4.0 International License

Introduction

Gamete quality is a determining factor for the success of bovine in vitro fertilization (IVF) (Khurana & Niemann, 2000; Walters et al., 2004). Several studies have confirmed the importance of the oocyte (Rahman et al., 2025), follicle size, cumulus-oocyte complex (COC) morphology, and the hormonal environment at the time of ovum pick-up on the resulting blastocyst rate (De Bem et al., 2014; Fair & Lonergan, 2023).

Similarly, sperm damage not only decreases motility and velocity, which are associated with reduced fertility potential (Li et al., 2016), but also increases DNA fragmentation, leading to lower fertilization rates and impaired embryo development (Mateo-Otero et al., 2022; Vallet-Buisan et al., 2023).

Consequently, employing semen collection strategies is necessary to rescue viable sperm when valuable genetics are available only in limited or low-viability samples (Oliveira et al., 2020). In IVF laboratories that use frozen semen, standard protocols must be adapted to recover the progressive subpopulation and achieve successful embryo production (Víquez et al., 2023).

Extensive research has focused on semen selection techniques aimed at rescuing low-viability samples, including swim-up, density gradient centrifugation, glass wool filtration, and Sephadex columns (Lee et al., 2009; Vaughan & Sakkas, 2019; Travnickova et al., 2021). However, no broad consensus currently exists regarding the most appropriate technique for processing compromised genetic material.

The objective of this work was to test different strategies to produce IVF blastocysts from low-viability bull semen.

Materials and Methods

The experiment encompassed two steps: the selection of the best IVF washing protocol in terms of blastocyst production using a pooled sample of low-viability semen, and the performance test of the selected IVF protocol on all low-viability semen samples.

Frozen semen samples from 10 Braford bulls were obtained from the Semen Laboratory within the Reproductive Biotechnology Laboratory at the Estación Experimental Agropecuaria Mercedes, part of the Instituto Nacional de Tecnología Agropecuaria, located in the Corrientes Regional Center, Argentina. The ovaries were provided by slaughterhouses and their use was authorized by the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA).

Semen straws were thawed at 37°C in a warm bath. A senior technician with 15 years of experience in semen evaluation subjectively analyzed the quality of the semen samples. The evaluated variables were the percentage of progressive motility immediately after thawing (H0), the percentage of progressive motility 2 hours after thawing (H2), vigor scored from 0 to 5, live spermatozoa (%), abnormalities (%), and the hypoosmotic swelling (HOS) test expressed as a percentage (Table 1). Vigor and abnormalities were determined using the method of Aller et al. (2012), whereas H0, H2, live spermatozoa, and HOS were determined using the method of Domínguez et al. (2008). Although no global consensus exists regarding the quality threshold for thawed semen, previous studies report that acceptable progressive motility semen should range between 55 and 65% (Kjaestad et al., 1993; Morrell et al., 2018). Given these criteria,

Table 1. Means and standard deviation of semen quality parameters evaluated in thawed semen collected from 10 bulls.

Bull[(1)]	H0 (%)	H2 (%)	Vigor	Live (%)	Normal (%)	HOS (%)
1	23 (1)	6 (8)	2.75 (0.5)	26 (3)	85 (2)	24 (8)
2	27 (7)	6 (4)	2.75 (0.1)	32 (12)	80 (2)	15 (1)
3	25 (1)	5 (2)	3.5 (0.6)	23 (5)	89 (2)	11 (2)
4	26 (2)	8 (9)	3.5 (0.6)	25 (5)	89 (2)	17 (6)
5	26 (1)	7 (4)	3.5 (1)	23 (4)	89 (1)	14 (5)
6	26 (5)	8 (5)	3.5 (1)	28 (5)	89 (1)	20 (5)
7	37 (2)	15 (13)	3.5 (0.6)	30 (11)	87 (3)	16 (7)
8	11 (7)	3 (1)	2.5 (0.6)	17 (5)	85 (2)	15 (4)
9	23 (2)	5 (6)	2.5 (0.6)	28 (1)	91 (1)	13 (4)
10	34 (1)	13 (8)	3.75 (0.5)	30 (7)	89 (4)	14 (4)

[(1)]H0, sperm motility immediately after thawning; H2, sperm motility 2 hours after thawning; and HOS, hypoosmotic swelling test.

the semen samples used in this study were classified as low-viability.

Five IVF dilution and separation protocols were evaluated, each carried out in two replicates. For each protocol, a pool of two semen samples from randomly selected bulls was used. The success rate was expressed as the percentage of blastocysts obtained relative to the total number of COCs. The COCs were obtained from ovaries provided by slaughterhouses, underwent in vitro maturation (IVM) for 24 hours at 35.8°C in a humidified atmosphere with a 6.5% CO_2 concentration. Finally, groups of 20 COCs were co-incubated with semen obtained from each protocol under the same atmospheric conditions used for IVM.

The IVF protocols included one control treatment and four washing protocols: swim-up, glass wool filtration, gradient-short IVF (SIVF), and gradient-long IVF (LIVF). The control treatment comprised frozen semen washed twice. Each washing involved a dilution process in sperm washing solution (SWS) followed by centrifugation at 490 × g for 5 min. Spermatozoa concentration was adjusted to 16×10^6 mL^{-1}, and co-incubated in Brackett and Oliphant (BO) media for 5 hours.

The swim-up washing protocol consisted of depositing 300 µL of semen at the bottom of 1 mL of SWS for 1 hour. After that, 800 µL of the supernatant were recovered, the spermatozoa concentration was adjusted to 1×10^6 mL^{-1}, and the sample was co-incubated in BO media for 5 hours.

The glass wool washing protocol consisted of column filtration as described by Lee et al. (2009). Following filtration, the spermatozoa concentration of the eluate was adjusted to 5×10^6 mL^{-1} before co-incubation in BO media for 5 hours.

The SIVF washing protocol consisted of layering a 40% density gradient solution on top of the 80% solution of colloidal medium (PureCeption, CooperSurgical, Ballerup, Denmark) in a single column to ensure distinct phase separation between both concentrations. Semen was deposited on the top layer and the column was centrifuged at 800 rpm for 10 min. A 200 aliquot recovered from the bottom of the column was re-suspended in 1 mL of SWS and centrifuged at 490 × g for 5 min. The resulting pellet was re-suspended to achieve a spermatozoa concentration of 1×10^6 mL^{-1} before co-incubation for 5 hours.

The LIVF washing protocol consisted a 40% density gradient solution on top of an 80% solution of colloidal medium (PureCeption, CooperSurgical, Ballerup, Denmark) in a single column. Semen was deposited on the top layer, and the column was centrifuged at 800 rpm for 10 min. A 200 µL aliquot recovered from the bottom of the column was re-suspended in 1 mL of Syntehtic Oviduct Fluid (SOF) medium supplemented with caffeine, and a modified version of regular SOF (Tervit et al., 1972). The suspension was then centrifuged at 490 × g for 5 min. The resulting pellet was re-suspended to achieve a spermatozoa concentration of 1×10^6 mL^{-1} before co-incubation for 20 hours.

For the second step of the experiment, the SIVF washing protocol was used because it yielded the highest blastocyst production success rate. Initially, six replicates were planned for each bull. As bull no. 1 presented poor semen recovery, two additional replicates were carried out for this animal, in a total of 62 replicates. Due to low viability of the semen samples, a single 100 µL drop of seminal suspension was used for each replicate. Nevertheless, in 10 replicates, it was possible to recover up to 150 µL of suspension. In those instances, two drops were used per replicate: one 100 µL drop co-incubated with 20 COCs, and a second 50 µL drop co-incubated with 10 COCs.

Presumptive zygotes were cultured in 50 µL droplets of SOF enriched with 2.5% fetal bovine serum under a 6.5% CO_2 concentration environment (Holm et al., 1999). Cleavage was evaluated 48 hours post-insemination via direct counting, and was calculated by dividing the number of cleaved embryos by the total number of presumptive zygotes. Concurrently, the developmental stage of each embryo was recorded by classifying them according to the blastomeres quantity (2, 4, 8, or 16 cells). For instance, the percentage of embryos at the 2-cell stage was calculated by dividing the number of 2-cell embryos by the total number of cleaved embryos. Finally, at day 7 of culture, the blastocyst rate was calculated as the number of observed blastocysts divided by the number of presumptive zygotes, and then the values were recorded.

Data was checked for normality and homoscedasticity using the modified Shapiro-Wilk and Levene's tests, respectively. Independence of errors and linearity were not checked. Because a correlation was observed between the cleavage and blastocyst rate variables, and the underlying biological

explanation for this correlation could not be identified among individual bulls, these variables were excluded from the analysis of variance (ANOVA). Data were adjusted using arcsine transformation and analyzed using the general linear model (GLM). When assumptions of normality and homoscedasticity were met, one-way ANOVA was performed using the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). To evaluate differences in embryo development among individual bulls, InfoStat 2020 (Di Rienzo et al., 2020) was used. The percentage results of cleavage and blastocyst were expressed as least square means and standard error, and mean comparisons were performed through Duncan's test ($\alpha = 0.05$).

Results and Discussion

Low-viability semen exhibited poor sperm motility at H0 and negligible motility at H2, indicating a high degree of cellular damage (Table 1). Such alterations are typically attributed to plasma membrane damage (Khalil et al., 2018), which impairs sperm motility and metabolism (Pini et al., 2018), and consequently, the ability of spermatozoa to interact with the female reproductive tract (Yeste, 2016). Although motility is a useful indicator of viability, the HOS test provides a more reliable assessment of membrane integrity; it detects sublethal damage that may otherwise remain undetected within a population of motile spermatozoa (Hecht & Jeyendran, 2022).

Several techniques, such as swim-up, density gradients, glass wool filtration, and Sephadex columns, have been developed to isolate viable spermatozoa (Lee et al., 2009; Arias et al., 2017; Vega-Hidalgo et al., 2022). In the present study, cleavage was observed across all IVF washing protocols. However, blastocysts were only obtained using the SIVF protocol, which yielded a 38% success rate (Table 2). Conversely, blastocysts were not observed in the control treatment, despite expectations. This outcome aligns with the findings of Arias et al. (2017), who observed that sperm selection through gradient techniques isolates a small population of highly motile spermatozoa capable of sustaining embryo development post-fertilization. Another reason for the SIVF success may be related to the BO medium used in this protocol. Specifically, it contained a higher caffeine concentration (4 mg mL^{-1}) than the BO medium used in LIVF (0.5 mg mL^{-1}). Caffeine markedly enhances sperm motility and velocity (Umezu et al., 2021).

Regarding the SIVF performance evaluation across individual semen samples, embryo development velocity differed among bulls (Figure 1). The number of embryos progressing beyond the 2-cell stage was significantly lower ($p<0.05$) in bulls no. 2 and no. 7 (Figure 1), an outcome consistent with their cleavage and blastocyst rates presented in Table 3. Conversely, cleavage rates were significantly higher ($p<0.05$) in bulls no. 6 and no. 9, whereas blastocyst rates were significantly higher ($p<0.05$) in bulls no. 1 and no. 6 (Table 3). While the SIVF protocol enabled blastocyst production from the majority of samples, failed to sustain embryogenesis, yielding a minimal 2% blastocyst rate with a 4% standard error, potentially due to a differential tolerance to stress (Table 3). These results highlight individual variations in response to the protocol. Such divergence has been associated with the lipid composition of sperm membranes, which contributes to the maintenance of structural stability during temperature fluctuations, thereby preventing organelle and DNA damage (Flores et al., 2011; Upadhyay et al., 2021). Notably, spermatozoa with fragmented DNA sometimes maintains motility and can successfully fertilize an oocyte (Li et al., 2016), which ultimately leads to impaired embryo development. This phenomenon was likely observed in the present study, as an increase of 2-cell blocked embryos was detected in two bulls (Borges Júnior et al., 2019; Mateo-Otero et al., 2022; Vallet-Buisan et al., 2023). These bulls exhibited 4 or fewer cells in 90% of their cleaved embryos, with more than 50% specifically at the 2-cell stage, showing underdevelopment.

Table 2. Means and standard deviation of cleavage and blastocyst production rates of cumulus-oocyte complexes resulted from low-viability semen subject to different washing protocols.

Protocol[(1)]	COCs	Cleavage (%)	Blastocyst (%)
Control	72	3 (4)	0 (0)
Swim up	47	3 (6)	0 (0)
Glass wool	44	7 (16)	0 (0)
SIVF	47	30 (64)	18 (38)
LIVF	43	8 (19)	1 (2)

[(1)]SIVF, gradient-short in vitro fertilization; LIVF, gradient-long in vitro fertilization; and COCs, cumulus-oocytes complexes.

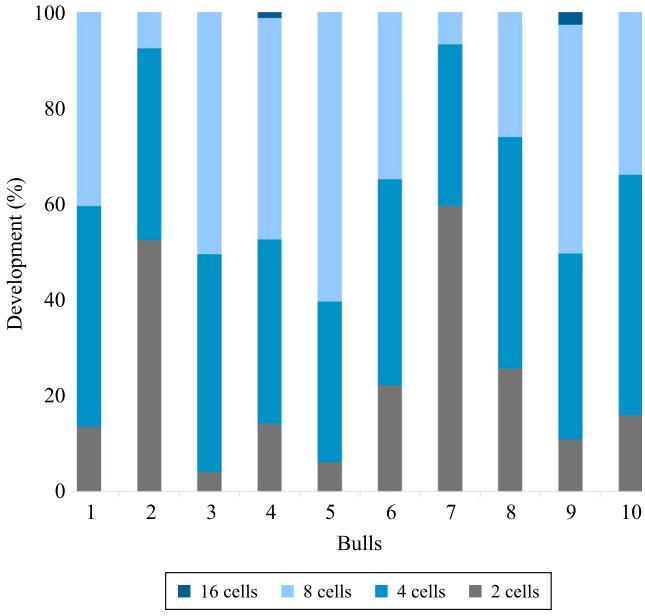

Figure 1. Distribution of embryonic developmental stages (2, 4, 8, and 16-cell embryos) at 48 hours post-insemination following the SIVF protocol using low-viability bull semen.

Table 3. Least square means and standard error for cleavage and blastocyst production rates of cumulus-oocytes complexes (COCs) fertilized with low-viability semen from individual bulls using the SIVF protocol[(1)].

Bull	COCs	Cleavage rate (%)	Blastocyst production (%)
1	150	47 ± 8ab	15 ± 4a
2	119	19 ± 9c	2 ± 4b
3	138	48 ± 9ab	14 ± 4ab
4	133	34 ± 9bc	6 ± 4ab
5	132	52 ± 9ab	14 ± 4ab
6	116	64 ± 9a	18 ± 4a
7	130	19 ± 9c	2 ± 4b
8	163	37 ± 9bc	13 ± 4ab
9	138	65 ± 9a	12 ± 4ab
10	119	53 ± 9ab	14 ± 4ab

[(1)]Means followed by different letters, in the columns, differ significantly by Duncan's test (α = 0.05).

Conclusions

1. The combination of gradient separation and short in vitro fertilization (SIVF) represents a viable strategy for successfully producing blastocysts from low-viability bull semen.
2. The success of the SIVF technique remains subject to the individual animal variability.

References

ALLER, J.F.; AGUILAR, D.; VERA, T.; ALMEIDA, G.P.; ALBERIO, R.H. Seasonal variation in sexual behavior, plasma testosterone and semen characteristics of Argentine Pampinta and Corriedale rams. **Spanish Journal of Agricultural Research**, v.10, p.345-352, 2012. DOI: https://doi.org/10.5424/sjar/2012102-389-11.

ARIAS, M.E.; ANDARA, K.; BRIONES, E.; FELMER, R. Bovine sperm separation by Swim-up and density gradients (Percoll and BoviPure): effect on sperm quality, function and gene expression. **Reproduction Biology**, v.17, p.126-132, 2017. DOI: https://doi.org/10.1016/j.repbio.2017.03.002.

BEM, T.H.C. de; ADONA, P.R.; BRESSAN, F.F.; MESQUITA, L.G.; CHIARATTI, M.R.; MEIRELLES, F.V.; LEAL, C.L.V. The influence of morphology, follicle size and Bcl-2 and Bax transcripts on the developmental competence of bovine oocytes. **Reproduction in Domestic Animals**, v.49, p.576-583, 2014. DOI: https://doi.org/10.1111/rda.12325.

BORGES JÚNIOR, E.; ZANETTI, B.F.; SETTI, A.S.; BRAGA, D.P. de A.F.; PROVENZA, R.R.; IACONELLI JÚNIOR, A. Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. **Fertility and Sterility**, v.112, p.483-490, 2019. DOI: https://doi.org/10.1016/j.fertnstert.2019.04.029.

DI RIENZO, J.A.; CASANOVES, F.; BALZARINI, M.; GONZALEZ, L.; TABLADA, M.; ROBLEDO, C.W. **InfoStat versión 2020**. Córdoba: Centro de Transferencia InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, 2020. Available at: <http://www.infostat.com.ar>. Accessed on: June 24 2026.

DOMÍNGUEZ, M.P.; FALCINELLI, A.; HOZBOR, F.; SÁNCHEZ, E.; CESARI, A.; ALBERIO, R.H. Seasonal variations in the composition of ram seminal plasma and its effect on frozen-thawed ram sperm. **Theriogenology**, v.69, p.564-573, 2008. DOI: https://doi.org/10.1016/j.theriogenology.2007.11.010.

FAIR, T.; LONERGAN, P. The oocyte: the key player in the success of assisted reproduction technologies. **Reproduction, Fertility and Development**, v.36, p.133-148, 2023. DOI: https://doi.org/10.1071/RD23164.

FLORES, E.; RAMIÓ-LLUCH, L.; BUCCI, D.; FERNÁNDEZ-NOVELL, J.M.; PEÑA, A.; RODRÍGUEZ-GIL, J.E. Freezing-thawing induces alterations in histone H1-DNA binding and the breaking of protein-DNA disulfide bonds in boar sperm. **Theriogenology**, v.76, p.1450-1464, 2011. DOI: https://doi.org/10.1016/j.theriogenology.2011.05.039.

HECHT, B.R.; JEYENDRAN, R.S. The hypo-osmotic swelling test: is it a sperm vitality or a viability assay? **F&S Science**, v.3, p.18-20, 2022. DOI: https://doi.org/10.1016/j.xfss.2021.11.001.

HOLM, P.; BOOTH, P.J.; SCHMIDT, M.H.; GREVE, T.; CALLESEN, H. High bovine blastocyst development in a static in vitro production system using sofaa medium supplemented

with sodium citrate and myo-inositol with or without serum-proteins. **Theriogenology**, v.52, p.683-700, 1999. DOI: https://doi.org/10.1016/S0093-691X(99)00162-4.

KHALIL, W.A.; EL-HARAIRY, M.A.; ZEIDAN, A.E.B.; HASSAN, M.A.E.; MOHEY-ELSAEED, O. Evaluation of bull spermatozoa during and after cryopreservation: structural and ultrastructural insights. **International Journal of Veterinary Science and Medicine**, v.6, p.S49-S56, 2018. Suppl.1. DOI: https://doi.org/10.1016/j.ijvsm.2017.11.001.

KHURANA, N.K.; NIEMANN, H. Effects of oocyte quality, oxygen tension, embryo density, cumulus cells and energy substrates on cleavage and morula/blastocyst formation of bovine embryos. **Theriogenology**, v.54, p.741-756, 2000. DOI: https://doi.org/10.1016/S0093-691X(00)00387-3.

KJAESTAD, H.; ROPSTAD, E.; ANDERSEN BERG, K. Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. **Acta Veterinaria Scandinavica**, v.34, p.299-303, 1993. DOI: https://doi.org/10.1186/BF03548194.

LEE, H.-L.; KIM, S.-H.; JI, D.-B.; KIM, Y.-J. A comparative study of Sephadex, glass wool and Percoll separation techniques on sperm quality and IVF results for cryopreserved bovine semen. **Journal of Veterinary Science**, v.10, p.249-255, 2009. DOI: https://doi.org/10.4142/jvs.2009.10.3.249.

LI, Y.; KALO, D.; ZERON, Y.; ROTH, Z. Progressive motility – a potential predictive parameter for semen fertilization capacity in bovines. **Zygote**, v.24, p.70-82, 2016. DOI: https://doi.org/10.1017/S0967199414000720.

MATEO-OTERO, Y.; LLAVANERA, M.; RECUERO, S.; DELGADO-BERMÚDEZ, A.; BARRANCO, I.; RIBAS-MAYNOU, J.; YESTE, M. Sperm DNA damage compromises embryo development, but not oocyte fertilisation in pigs. **Biological Research**, v.55, art.15, 2022. DOI: https://doi.org/10.1186/s40659-022-00386-2.

MORRELL, J.M.; VALEANU, A.S.; LUNDEHEIM, N.; JOHANNISSON, A. Sperm quality in frozen beef and dairy bull semen. **Acta Veterinaria Scandinavica**, v.60, art.41, 2018. DOI: https://doi.org/10.1186/s13028-018-0396-2.

OLIVEIRA, S.N.; ANDRADE JR., L.R.P.; SILVA, L.F.M.C.; ARAUJO, E.A.B.; RAYASHI, R.M.; SEGABINAZZI, L.G.T.M.; ALVARENGA, M.A.; DELL'AQUA, C.P.F.; DELL'AQUA JR., J.A.; PAPA, F.O. Fractionated semen collection as a tool to rescue fertility in stallions with seminal vesiculitis. **Theriogenology**, v.157, p.110-120, 2020. DOI: https://doi.org/10.1016/j.theriogenology.2020.07.014.

PINI, T.; LEAHY, T.; DE GRAAF, S.P. Sublethal sperm freezing damage: manifestations and solutions. **Theriogenology**, v.118, p.172-181, 2018. DOI: https://doi.org/10.1016/j.theriogenology.2018.06.006.

RAHMAN, M.B.; HELD-HOELKER, E.; SAEED-ZIDANE, M.; RINGS, F.; SALILEW-WONDIM, D.; TESFAYE, D.; GAD, A.; GEBREMEDHN, S.; THOLEN, E.; SCHELLANDER, K.; GROßE-BRINKHAUS, C.; HOELKER, M. Comparative transcriptome analysis of bovine blastocysts reveals specific effects of the oocyte source and the environments during maturation and early embryo development. **BMC Genomics**, v.26, art.673, 2025. DOI: https://doi.org/10.1186/s12864-025-11848-8.

TERVIT, H.R.; WHITTINGHAM, D.G.; ROWSON, L.E.A. Successful culture *in vitro* of sheep and cattle ova. **Journal of Reproduction and Fertility**, v.30, p.493-497, 1972. DOI: https://doi.org/10.1530/jrf.0.0300493.

TRAVNICKOVA, I.; HULINSKA, P.; KUBICKOVA, S.; HANZALOVA, K.; KEMPISTY, B.; NEMCOVA, L.; MACHATKOVA, M. Production of sexed bovine embryos in vitro can be improved by selection of sperm treatment and co-culture system. **Reproduction in Domestic Animals**, v.56, p.864-871, 2021. DOI: https://doi.org/10.1111/rda.13926.

UMEZU, K.; KURATA, S.; HARA, K.; TANEMURA, K. Caffeine induces sperm detachment from sperm head-to-head agglutination in bull. **Biochemical and Biophysical Research Communications,** v.562, p.105-111, 2021. DOI: https://doi.org/10.1016/j.bbrc.2021.05.060.

UPADHYAY, V.R.; RAMESH, V.; DEWRY, R.K.; KUMAR, G.; RAVAL, K.; PATOLIYA, P. Implications of cryopreservation on structural and functional attributes of bovine spermatozoa: an overview. **Andrologia**, v.53, e14154, 2021. DOI: https://doi.org/10.1111/and.14154.

VALLET-BUISAN, M.; MECCA, R.; JONES, C.; COWARD, K.; YESTE, M. Contribution of semen to early embryo development: fertilization and beyond. **Human Reproduction Update**, v.29, p.395-433, 2023. DOI: https://doi.org/10.1093/humupd/dmad006.

VAUGHAN, D.A.; SAKKAS, D. Sperm selection methods in the 21st century. **Biology of Reproduction**, v.101, p.1076-1082, 2019. DOI: https://doi.org/10.1093/biolre/ioz032.

VEGA-HIDALGO, J.; RODRIGUEZ, M.; DIPAZ-BERROCAL, D.; RIVAS, J.; HUAYHUA, C.; MELLISHO, E. Sperm selection techniques in cattle: microfilter device versus conventional methods. **Andrologia**, v.54, e14585, 2022. DOI: https://doi.org/10.1111/and.14585.

VÍQUEZ, L.; SEVILLA, F.; ARAYA-ZÚÑIGA, I.; SOLER, C.; BARQUERO, V.; ROLDAN, E.R.S.; VALVERDE, A. Morphometric assessment of cryopreserved livestock bull spermatozoa in the tropics. **Reproduction in Domestic Animals**, v.58, p.1439-1447, 2023. DOI: https://doi.org/10.1111/rda.14459.

WALTERS, A.H.; EYESTONE, W.E.; SAACKE, R.G.; PEARSON, R.E.; GWAZDAUSKAS, F.C. Sperm morphology and preparation method affect bovine embryonic development. **Journal of Andrology**, v.25, p.554-563, 2004. DOI: https://doi.org/10.1002/j.1939-4640.2004.tb02826.x.

YESTE, M. Sperm cryopreservation update: cryodamage, markers, and factors affecting the sperm freezability in pigs. **Theriogenology**, v.85, p.47-64. 2016. DOI: https://doi.org/10.1016/j.theriogenology.2015.09.047.

Author contributions

Franco Alejandro Dellavalle: conceptualization (equal), formal analysis (equal), investigation (equal), methodology (equal), project administration (equal), writing – original draft (equal); **Andres Rogberg-Muñoz**: conceptualization (equal), formal analysis (equal), funding acquisition (equal), investigation (equal), supervision (equal), writing – review & editing (equal); **Juan Manuel Benitez**: methodology (equal); **Domingo Emilio Aguilar**: methodology (equal); **Rafael Fernandez-Martin**: conceptualization (equal), funding acquisition (equal), writing – review & editing (equal); **Amada Eugenia Ynsaurralde-Rivolta**: conceptualization (equal), formal analysis (equal), funding acquisition (equal), investigation (equal), methodology (equal), project administration (equal), writing – review & editing (equal).

Chief editor: Edemar Corazza
Edited by: Daniel Kinpara

Data availability statement

Data available upon request: research data are only available upon reasonable request to the corresponding author.

Declaration of use of AI technologies

No generative artificial intelligence (AI) was used in this study.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

To Asociación Cooperadora de la EEA de INTA Mercedes, for the funding support; and to Matarifes de Mercedes SA, for providing the ovaries for this study.

Disclaimer/Publisher's note

The statements, opinions, and data contained in all texts published in Pesquisa Agropecuária Brasileira (PAB) are solely those of the individual author(s) and not of the journal's publisher, editor, and editorial team, who disclaim responsibility for any injury to people or property resulting from any referred ideas, methods, instructions, or products.

The mention of specific chemical products, machines, and commercial equipment in the texts published in this journal does not imply their recommendation by the publisher.