CHALLENGES AND OPPORTUNITIES IN THE APPLICATION OF ARTIFICIAL INSEMINATION IN SHEEP BREEDING

Tudor POPA¹, Ellda Mellisa SAVU¹, Raluca-Aniela GHEORGHE-IRIMIA¹, Dana TĂPĂLOAGĂ¹, Cosmin ŞONEA¹, Makki Khalaf Hussein AL DULAIMI³, Eugen Adrian CHISA⁴, Paul-Rodian TĂPĂLOAGĂ²

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independenței, District 5, 050097, Bucharest, Romania
 ²University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Productions Engineering and Management, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania

³Al-Furat Al-Awsat Technical University, Baghdad, Iraq ⁴Technologic High School "Vintilă Brătianu", Dragomiresti Vale, Romania

Corresponding author email: raluca.irimia@fmv.usamv.ro

Abstract

The use of artificial insemination (AI) in sheep breeding poses both considerable obstacles and exciting prospects. This research delves into the complexities of artificial insemination, focussing on factors influencing fertility rates, semen handling techniques, and the effect of hormone treatments on reproductive success. Several studies reveal that the success of AI is dependent on ideal sperm quality, which can be negatively impacted by cryopreservation procedures; for example, the concentration of sperm upon freezing has been found to influence post-thaw quality and subsequent reproductive outcomes in sheep. Furthermore, the method of insemination used - whether cervical or laparoscopic - is important, with laparoscopic techniques frequently providing greater pregnancy rates due to their ability to avoid anatomical challenges presented by the ewe's cervix. Furthermore, synchronising oestrus with hormonal therapies, such as oxytocin or equine chorionic gonadotropin (eCG), has been found as a critical element in improving the timing and efficiency of AI. However, the unpredictability in conception rates is still a problem, with reported statistics ranging from 20% to 70% depending on the procedures used and the settings under which AI is conducted. Environmental influences, such as temperature and stress, impair reproductive results, especially in the vital early phases of embryo development. The aim of this review is to summarise current research in order to identify best practices and new opportunities for improving the efficacy of AI in sheep breeding.

Key words: artificial insemination, Fertility Rates, sheep breeding.

INTRODUCTION

Artificial insemination (AI) is an important reproductive tool in livestock breeding, enabling the genetic enhancement of multiple species, including sheep. The technique entails the deliberate introduction of sperm into a female's reproductive tract to facilitate conception without natural mating. AI has transformed livestock breeding by facilitating the swift distribution of better genetics, therefore improving production and genetic diversity in herds. The efficacy of artificial insemination is dependent on parameters, including semen quality, the time of insemination in relation to the female's oestrus cycle, and the skill level of the inseminator (Paul, 2024; Sitepu et al., 2023; Faigl et al., 2012). Artificial insemination is pivotal in sheep breeding for genetic advancement and productivity advancement. The use of AI facilitates use of genetically superior rams, hence significantly enhancing the genetic quality of the offspring. This is particularly important in ovine husbandry, where conventional breeding techniques may vield restricted genetic diversity diminished genetic advancement. Through the application of AI, breeders can more swiftly include desirable traits, including enhanced wool quality, disease resistance, and superior reproductive performance (Sitepu et al., 2023; Faigl et al., 2012). Additionally, AI can mitigate inbreeding concerns by allowing the introduction of genetic material from distant populations, hence enhancing the overall

genetic diversity (Khandoker, 2023; Putri et al., 2021). Research indicates that pregnancy rates linked to artificial insemination in sheep may fluctuate, although under optimal settings, they attain considerable levels. Artificial insemination pregnancy rates in sheep can vary from 26% to 61%, influenced by factors including the timing of insemination and the quality of the frozen semen utilised (Sitepu et al., 2023; Jha et al., 2020). The adoption of fixed-time artificial insemination procedures has improved the efficacy of artificial insemination by synchronising oestrus in ewes, facilitating more accurate insemination timing and potentially elevating conception rates (Negreiros et al., 2020; McCappin & Murray, 2011). The economic ramifications of AI in sheep breeding are significant. The costeffectiveness of artificial insemination relative to natural mating is significant, as it diminishes the necessity for maintaining numerous rams and facilitates the utilisation of superior frozen semen from elite rams, which can be transported over extensive distances without compromising viability (Asaduzzaman et al., 2021). This not only reduces operational expenses but also allows farmers to obtain superior genetics that would otherwise be inaccessible in their local breeding populations (Paul, 2024; Asaduzzaman et al., 2021; Siti & Rachmawati, 2024; Faigl et al., 2012). Besides genetic and economic advantages, AI also enhances the health and welfare of sheep populations. AI diminishes the necessity for physical interaction among breeding animals, hence mitigating the possibility of disease transmission, a critical consideration in densely populated agricultural settings (Morrell et al., 2022; Faigl et al., 2012). Moreover, the capacity to select for particular features via AI can result in healthier and more resilient sheep, capable of thriving in various environmental conditions (Paul, 2024; Putri et al., 2021; Sarini et al., 2023; Musriati et al., 2024; Sirajuddin et al., 2018). The implementation of modern technology. including laparoscopic insemination procedures, has been proven to further improve conception rates, underscoring the necessity for constant improvement and adaptability in AI methodologies (McCappin & Murray, 2011; Faigl et al., 2012).

The aim of this review is to analyse the challenges and opportunities associated with artificial insemination in sheep breeding by evaluating factors affecting fertility, semen handling techniques, insemination methods, and hormonal synchronization.

MATERIALS AND METHODS

The materials for this review were collected through a systematic search of academic databases such as PubMed, ScienceDirect, and Google Scholar, using key words such as "artificial insemination in sheep", "semen handling techniques", and "fertility factors in ovine reproduction". The selected papers were evaluated based on their relevance, publication date, and methodology and the retrieved data were compared to highlight the field's challenges and potential.

RESULTS AND DISCUSSIONS

Factors influencing AI success in sheep Semen quality and handling techniques

Sperm quality is a crucial factor influencing reproduction results in various including livestock and humans. High-quality sperm, defined by superior motility, morphology, and membrane integrity, is positively associated with successful fertilisation rates (Zeng et al., 2021; Elmagd et al., 2022). Research indicates that sperm motility is a crucial predictor of fertility, with enhanced mass sperm motility correlating with elevated fertility rates in sheep (David et al., 2015). The integrity of DNA is crucial, since fragmentation negatively impact can fertilisation and subsequent potential embryonic development (González-Marín et al., 2012). Cryopreservation substantially affects sperm viability, frequently resulting in diminished motility and vitality after thawing. The freezing process can cause oxidative stress and lipid peroxidation, undermining sperm membrane integrity and functionality (Perumal et al., 2010; Jiménez-Rabadán et al., 2016). Studies demonstrate that including antioxidants in the cryopreservation process can alleviate adverse effects, hence improving post-thaw sperm quality (Perumal et al., 2010; Perumal et al., 2013). The concentration of sperm during freezing is critical; elevated concentrations can increase the probability of preserving viable sperm after thawing, thereby improving reproductive results (Madeddu et al., 2024).

Insemination methods

Cervical and laparoscopic inseminations are techniques for artificial common insemination (AI) in sheep, each possessing unique benefits and drawbacks. Cervical insemination (CAI) is less intrusive and more economical, making it accessible to numerous breeders. The anatomical limitations of the ewe's cervix, marked by its small and convoluted configuration, frequently limit efficient semen deposition, resulting in reduced conception rates, generally under 30% with frozen-thawed semen (Richardson et al., 2019; Álvarez al., 2019). Laparoscopic et facilitates insemination direct (LAI) intrauterine semen deposition, circumventing cervical barriers, which may significantly enhance fertility outcomes, especially with suboptimal sperm quality (El-Badry, 2014; Sathe, 2018). Research indicates that LAI can attain conception rates over 70% under ideal circumstances (Masoudi et al., 2017; McCappin & Murray, 2011). Various strategies can be utilised to enhance insemination efficiency. Synchronisation methods. including progesterone-CIDR, can optimise ovulation time and augment conception rates (Moreira et al., 2023; Álvarez et al., 2019). Furthermore, maintaining high-quality sperm via appropriate cryopreservation methods and oxidative stress during storage are essential for optimising post-thaw viability (Bülbül et al., 2024). Moreover, innovations in transcervical methods, including the surgical excision of cervical folds, could enhance accessibility and elevate pregnancy rates (Pau et al., 2020). Although LAI presents enhanced fertility potential, the decision between CAI and LAI must take into account aspects such as expense, necessary skills, and particular breeding objectives.

Hormonal synchronization and fertility regulation

Hormonal therapies, such equine chorionic gonadotropin (eCG) and oxytocin, are crucial for oestrus synchronisation in sheep. eCG is

frequently employed to promote follicular maturation and induce oestrus, whilst oxytocin can aid in uterine contractions and boost reproductive results after insemination (Silva et al., 2020; Souza et al., 2021). The efficacy of these hormonal treatments can result in synchronised oestrus, facilitating enhanced breeding control and elevated lambing rates (Souza et al., 2021). The influence of hormone regimens on reproductive outcomes significant. Research indicates administration of eCG alongside progestogens can markedly enhance the proportion of ewes displaying oestrus and increase the conception rates (Mohan, 2023; Ouintero-Elisea et al., 2011). Hormonal therapies can prolong and intensify oestrus, which are essential for successful mating (Mohan, 2023). Nonetheless, fluctuations in conception rates may arise depending on the synchronisation techniques utilised. Research demonstrates that certain treatments attain high synchronisation rates (up to 100%), whilst others may produce lower rates influenced by factors such as individual animal responses, ambient conditions, and nutritional status (Souza et al., 2021; Wu et al., 2022).

Challenges and future directions

Regarding challenges, heat stress significantly impacts fertility and embryonic development in livestock, especially in sheep and cattle. Increased temperatures can negatively impact oocyte maturation, fertilisation, and subsequent embryonic development. Research indicates that exposure to temperatures between 39.5-41.0°C during the initial phases of in vitro culture (IVC) can diminish the growth rates of bovine embryos, resulting in decreased blastocyst formation rates (Alves et al., 2013; Namekawa et al., 2010). Moreover, heat stress can interfere with follicular dynamics, leading to a decrease in viable follicles and diminished reproductive success (Ratchamak et al., 2022; Ratchamak et al., 2021).

Proper nutrition reduces certain adverse effects of heat stress by enhancing metabolic processes and preserving overall health (Feugere et al., 2022; Ortega et al., 2016). Effective management strategies, including the provision of shade, sufficient water, and nutritional supplements, can bolster resilience to heat

and promote reproduction rates (Ratchamak et al., 2021; Crews et al., 2015). Implementing stress reduction measures is crucial for enhancing artificial insemination (AI) results. Methods include environmental interventions. changes dietary administrations of stress-relieving supplements such as melatonin have demonstrated potential reproductive enhancing efficiency (Ratchamak et al., 2022; Ortega et al., 2016). Producers can improve the reproductive performance of their flocks by addressing environmental and dietary issues, especially during heat stress periods.

The future of artificial insemination (AI) in sheep is set for substantial progress, especially in semen preservation methods, laparoscopic technology, genetic selection. comprehensive optimisation methodologies. Innovations in cryopreservation procedures, such as the use of novel cryoprotectants and antioxidants, are essential for improving the viability of frozen-thawed semen. Recent studies indicate that the use of antioxidants during freezing can reduce oxidative stress, enhancing post-thaw motility and fertility rates (Pelayo et al., 2019; Yang, 2024). The investigation of different cryoprotectants may improve sperm viability during freezing and thawing, resulting in superior outcomes in artificial insemination programs (Akter et al., 2020).

Laparoscopic AI has already exhibited higher conception rates compared to conventional cervical insemination. Future advancements may concentrate on improving laparoscopic procedures to diminish invasiveness and facilitate use, possibly integrating real-time imaging technology to augment precision during insemination (Akter et al., 2020). Investigating the optimisation of insemination timing in relation to ovulation is essential, especially when utilising frozen-thawed semen, which has a restricted viability period (Akter et al., 2020). The genetic selection of traits that improve compatibility with AI is an increasing area of interest. Identifying and choosing genetic markers linked to reproductive efficiency, semen quality, and fertility can substantially enhance flock productivity (Pelayo et al., 2019; Yang, 2024; Nosrati et al., 2018; Gheorghe-Irimia, 2023). Genome-wide association studies (GWAS) have identified candidate genes associated with reproductive traits, which can guide breeding programs to improve artificial insemination success rates (Yang, 2024; Nosrati et al., 2018). Ongoing research is crucial for enhancing AI procedures. This involves examining the impact of environmental variables, such as thermal stress. reproductive efficacy and nutritional approaches that may improve fertility during artificial insemination (Narayan et al., 2018: Boareki et al., 2021: Gheorghe-Irimia, 2024; Sonea et al., 2023a; Sonea et al., 2023b). Furthermore, the combination of genomic selection with conventional breeding methodologies might accelerate genetic advancements and enhance the general resilience of sheep populations (Zhang et al., 2023; Li et al., 2020).

CONCLUSIONS

Artificial insemination (AI) has significantly progressed sheep breeding by facilitating genetic enhancement, boosting productivity, and augmenting genetic diversity. Despite all of its advantages, AI encounters significant obstacles, including fluctuations in conception rates, deterioration of semen quality owing to cryopreservation, and the complicated nature of insemination methodologies. The implementation of hormonal synchronisation insemination enhanced techniques, especially laparoscopic artificial insemination, has facilitated the optimisation of conception rates; yet environmental and management aspects remain pivotal to reproductive success. Continuous research is essential for improving semen preservation methods. advancing artificial insemination procedures, and discovering genetic markers linked to reproductive effectiveness. Moreover, mitigating environmental stresses optimising nutritional management can further improve AI results in sheep breeding. Through the integration of technical improvements and optimal management techniques, AI can remain a crucial instrument for sustainable and effective sheep reproduction, guaranteeing economic viability and genetic advancement within the industry.

REFERENCES

- Akter, S., Asaduzzaman, M., Saha, A., Alam, M., & Bari, F. (2020). Factors affecting the pregnancy rate of Bangladeshi ewes following laparoscopic artificial insemination (LAP-AI). *Veterinary Integrative Sciences*, 20(2), 309-316. https://doi.org/10.12982/vis.2022.024
- Álvarez, M., Anel-López, L., Boixo, J., Chamorro, C., Neila-Montero, M., Montes-Garrido, R.& Anel, L. (2019). Current challenges in sheep artificial insemination: A particular insight. *Reproduction in Domestic Animals*, 54(S4), 32-40. https://doi.org/10.1111/rda.13523
- Alves, M., Gonçalves, R., Pavão, D., Palazzi, E., Souza, F., Queiroz, R., & Achilles, M. (2013). Effect of heat stress on the maturation, fertilization, and development rates of *in vitro* produced bovine embryos. *Open Journal of Animal Sciences*, 3(3), 174-178. https://doi.org/10.4236/ojas.2013.33026
- Asaduzzaman, M., Saha, A., Alam, M., & Bari, F. (2021). Developing a cost structure of frozen semen production and performance of artificial insemination for sheep breeding programs. *Agricultural Science Digest A Research Journal*. https://doi.org/10.18805/ag.d-321
- Boareki, M., Schenkel, F., Willoughby, O., Suárez-Vega, A., Kennedy, D., & Cánovas, Á. (2021). Comparison between methods for measuring fecal egg count and estimating genetic parameters for gastrointestinal parasite resistance traits in sheep. *Journal of Animal Science*, 99(12). https://doi.org/10.1093/jas/skab341
- Bülbül, B., Dayanikli, C., Şengül, E., Turan, A., Aksoy, M., & Nur, Z. (2024). Failure of Isoxsuprine HCl to increase sheep fertility after cervical versus laparoscopic AI using chilled semen at different durations. *Animal Science Journal*, 95(1). https://doi.org/10.1111/asj.13973
- Crews, S., McCleery, W., & Hutson, M. (2015). Pathway to a phenocopy: Heat stress effects in early embryogenesis. *Developmental Dynamics*, 245(3), 402-413. https://doi.org/10.1002/dvdv.24360
- David, I., Kohnke, P., Lagriffoul, G., Praud, O., Plouarboué, F., Degond, P.,& Druart, X. (2015). Mass sperm motility is associated with fertility in sheep. *Animal Reproduction Science*, 161, 75-81. https://doi.org/10.1016/j.anireprosci.2015.08.006
- El-Badry, D.A., Leil, A.Z., & Shaker, M.H. (2014). Studies on laparoscopic intrauterine insemination of Barki ewes using different insemination doses as compared with cervical insemination. Assiut Veterinary Medical Journal, 60(142), 172-178. https://doi.org/10.21608/avmj.2014.171004
- Elmagd, H., Sarhan, M., & Abu-Elnaga, N. (2022). A modified ICSI technique: Using zona pellucida as a natural bait. *The Egyptian Journal of Hospital Medicine*, 89(1), 5075-5082. https://doi.org/10.21608/ejhm.2022.261173
- Faigl, V., Vass, N., Jávor, A., Kulcsár, M., Solti, L., Amiridis, G. & Cseh, S. (2012). Artificial insemination of small ruminants - A review. Acta

- Veterinaria Hungarica, 60(1), 115-129. https://doi.org/10.1556/avet.2012.010
- Feugere, L., Bates, A., Emagbetere, T., Chapman, E., Malcolm, L., Bulmer, K., & Valero, K. (2022). Heat induces multi-omic and phenotypic stress propagation in zebrafish embryos. *PNAS Nexus*, 2(5), pgad137 https://doi.org/10.1101/2022.09.15.508176
- Gheorghe-Irimia, R.A., Sonea, C., Tăpăloagă, D., Gurau, M.R., Ilie, L.I., & Tăpăloagă, P.R. (2023). Innovations in dairy cattle management: Enhancing productivity and environmental sustainability. Annals of "Valahia" University of Târgovişte. Agriculture, 15(2), 18-25.
- Gheorghe-Irimia, R.A., Şonea, C., Udrea, L., Tăpăloagă, P.R., & Tăpăloagă, D. (2024). The nexus between animal nutrition, health, and environmental sustainability in rural areas. Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Science and Food Science and Technology, 23(A), 285–293.
- González-Marín, C., Gosálvez, J., & Roy, R. (2012). Types, causes, detection, and repair of DNA fragmentation in animal and human sperm cells. *International Journal of Molecular Sciences*, 13(11), 14026-14052. https://doi.org/10.3390/ijms131114026
- Jha, P., Alam, M., Mansur, M., Talukder, M., Naher, N., Rahman, A.& Bari, F. (2020). Effects of number of frozen-thawed ram sperm and number of inseminations on fertility in synchronized ewes under field conditions. *Journal of Animal Reproduction and Biotechnology*, 35(2), 190-197. https://doi.org/10.12750/jarb.35.2.190
- Jiménez-Rabadán, P., Soler, A., Ramón, M., García-Álvarez, O., Maroto-Morales, A., Iniesta-Cuerda, M., & Garde, J. (2016). Influence of semen collection method on sperm cryoresistance in small ruminants. *Animal Reproduction Science*, 167, 103-108. https://doi.org/10.1016/j.anireprosci.2016.02.013
- Khandoker, M. (2023). Population distribution and breeding practices of livestock in different districts of Bangladesh. Asian-Australasian Journal of Bioscience and Biotechnology, 8(3), 38-48. https://doi.org/10.3329/aajbb.v8i3.67698
- Li, X., Yang, J., Shen, M., Xie, X., Liu, G., Xu, Y. & Li, M. (2020). Whole-genome resequencing of wild and domestic sheep identifies genes associated with morphological and agronomic traits. *Nature Communications*, 11(1) https://doi.org/10.1038/s41467-020-16485-1
- Madeddu, M., Zaniboni, L., Marelli, S., Tognoli, C.,
 Belcredito, S., Iaffaldano, N. & Cerolini, S. (2024).
 Selection of male donors in local chicken breeds to implement the Italian semen cryobank: Variability in semen quality, freezability, and fertility. *Veterinary Sciences*, 11(4), 148.
 https://doi.org/10.3390/vetsci11040148
- Masoudi, R., Shahneh, A., Towhidi, A., Kohram, H., Akbarisharif, A., & Sharafi, M. (2017). Fertility response of artificial insemination methods in sheep with fresh and frozen-thawed semen. *Cryobiology*, 74, 77-80
 - https://doi.org/10.1016/j.cryobiol.2016.11.012

- McCappin, N., & Murray, R. (2011). Some factors affecting pregnancy rate in ewes following laparoscopic artificial insemination. *Veterinary Record*, 168(4), 99-99. https://doi.org/10.1136/vr.c5979
- Mohan, K. (2023). Comparative evaluation of estrus synchronization protocols on reproductive performance and estrus behavior in Barbados Black Belly sheep. *Veterinary World*, *16*(9), 2244-2249. https://doi.org/10.14202/vetworld.2023.2244-2249
- Moreira, N., Brasil, O., Conceição, F., Oliveira, R., & Ramos, A. (2023). Short protocol with progesterone-CIDR shows high ovulation synchrony and high fertility following artificial insemination in hair sheep. Research Square, https://doi.org/10.21203/rs.3.rs-2729531/v1
- Morrell, J., Malaluang, P., Cojkic, A., & Hansson, I. (2022). Alternatives to antibiotics in semen extenders used in artificial insemination. *Pathogens*, 3(4), 934-946. https://doi.org/10.5772/intechopen.104226
- Musriati, A., Setiadi, A., & Samsudewa, D. (2024).

 Analysis of factors affecting the success of beef cattle artificial insemination (AI) in Jepon District, Blora Regency. *IOP Conference Series: Earth and Environmental Science*, 1364(1), 012038. https://doi.org/10.1088/1755-1315/1364/1/012038
- Namekawa, T., Ikeda, S., Sugimoto, M., & Kume, S. (2010). Effects of astaxanthin-containing oil on development and stress-related gene expression of bovine embryos exposed to heat stress. *Reproduction in Domestic Animals*, 45(6), 999-1005. https://doi.org/10.1111/j.1439-0531.2010.01584.x
- Narayan, E., Sawyer, G., & Parisella, S. (2018). Faecal glucocorticoid metabolites and body temperature in Australian Merino ewes (*Ovis aries*) during a summer artificial insemination (AI) program. *PLOS ONE*, 13(1), e0191961. https://doi.org/10.1371/journal.pone.0191961
- Negreiros, M., Seugling, G., Almeida, A., Hidalgo, M., Martins, M., Blaschi, W. & Barreiros, T. (2020). Influence of nutritional and ovarian parameters on pregnancy rates of Nelore cows artificially inseminated at fixed time. *Research Society and Development*, 9(9), e907998091. https://doi.org/10.33448/rsd-v9i9.8091
- Nosrati, M., Nanaei, H., Ghanatsaman, Z., & Esmailizadeh, A. (2018). Whole genome sequence analysis to detect signatures of positive selection for high fecundity in sheep. *Reproduction in Domestic Animals*, 54(2), 358-364. https://doi.org/10.1111/rda.13368
- Ortega, M., Rocha-Frigoni, N., Mingoti, G., Roth, Z., & Hansen, P. (2016). Modification of embryonic resistance to heat shock in cattle by melatonin and genetic variation in *HSPA1L. Journal of Dairy Science*, 99(11), 9152-9164. https://doi.org/10.3168/jds.2016-11501
- Pau, S., Falchi, L., Ledda, M., Pivato, I., Melosu, V., Bogliolo, L. & Zedda, M. (2020). Reproductive performance following transcervical insemination with frozen-thawed semen in ewes submitted to surgical incision of cervical folds (SICF): Comparison with laparoscopic artificial insemination.

- *Animals*, 10(1), 108. https://doi.org/10.3390/ani10010108
- Paul, A. (2024). Constraints and recommendation for countrywide extension of artificial insemination in buffalo, goat, and sheep. *Bangladesh Journal of Veterinary Medicine*, 21(2), 79-84. https://doi.org/10.33109/bjymjd2023fam4
- Pelayo, R., Ramón, M., Granado-Tajada, I., Ugarte, E., Serrano, M., Gutiérrez-Gil, B. & Arranz, J. (2019). Estimation of the genetic parameters for semen traits in Spanish dairy sheep. *Animals*, 9(12), 1147. https://doi.org/10.3390/ani9121147
- Perumal, P., Selvaraju, S., Selvakumar, S., Barik, A., Mohanty, D., Das, S. & Mishra, P. (2010). Effect of pre-freeze addition of cysteine hydrochloride and reduced glutathione in semen of crossbred Jersey bulls on sperm parameters and conception rates. *Reproduction in Domestic Animals*, 46(4), 636-641. https://doi.org/10.1111/j.1439-0531.2010.01719.x
- Perumal, P., Vüpru, K., & Khate, K. (2013). Effect of addition of melatonin on the liquid storage (5°C) of Mithun (*Bos frontalis*) semen. *International Journal of Zoology*, 2013, 1-10. https://doi.org/10.1155/2013/642632
- Putri, C., & Poetranto, E. (2021). The effect of the different artificial insemination time periods on the pregnancy rate of Sapudi ewes. World's Veterinary Journal, 11(3), 469-473. https://doi.org/10.54203/scil.2021.wvj60
- Quintero-Elisea, J., Macías Cruz, U., Álvarez-Valenzuela, F., Correa Calderón, A., González-Reyna, A., Lucero-Magaña, F.& Avendaño-Reyes, L. (2011). The effects of time and dose of pregnant mare serum gonadotropin (PMSG) on reproductive efficiency in hair sheep ewes. *Tropical Animal Health and Production*, 43(8), 1567-1573. https://doi.org/10.1007/s11250-011-9843-z
- Ratchamak, R., Ratsiri, T., Chumchai, R., Boonkum, W., & Chankitisakul, V. (2021). Relationship of the temperature-humidity index (THI) with ovarian responses and embryo production in superovulated Thai-Holstein crossbreds under tropical climate conditions. *Veterinary Sciences*, 8(11), 270. https://doi.org/10.3390/vetsci8110270
- Ratchamak, R., Thananurak, P., Boonkum, W., Semaming, Y., & Chankitisakul, V. (2022). The melatonin treatment improves the ovarian responses after superstimulation in Thai-Holstein crossbreeds under heat stress conditions. Frontiers in Veterinary Science, 9, 888039. https://doi.org/10.3389/fvets.2022.888039
- Richardson, L., Hanrahan, J., Tharmalingam, T., Carrington, S., Lonergan, P., Evans, A., & Fair, S. (2019). Cervical mucus sialic acid content determines the ability of frozen-thawed ram sperm to migrate through the cervix. *Reproduction*, 157(3), 259-271. https://doi.org/10.1530/rep-18-0547
- Sarini, N., Setyani, N., Suryani, N., & Suarna, I. (2023).

 Cattle inseminators profile in Bali Province.

 International Journal of Innovative Research in

 Multidisciplinary Education, 2(10).

 https://doi.org/10.58806/ijirme.2023.v2i10n10

- Sathe, S. (2018). Laparoscopic artificial insemination technique in small ruminants - A procedure review. Frontiers in Veterinary Science, 5, 266. https://doi.org/10.3389/fyets.2018.00266
- Silva, R., Oliveira, R., Silva, A., Oliveira, F., Rufino, J., & Silva, M. (2020). Effect of different protocols for estrus synchronization on reproductive performance of Santa Inês ewes under Amazon environmental conditions. Acta Scientiarum Animal Sciences, 43, e48954.
 - https://doi.org/10.4025/actascianimsci.v43i1.48954
- Sirajuddin, S., Sudirman, I., & Bahar, L. (2018). Relationship between breeder characteristics and adoption of artificial insemination in Bali cattle. European Journal of Sustainable Development, 7(3), 143-154.
 - https://doi.org/10.14207/ejsd.2018.v7n3p143
- Sitepu, S., Kurniawan, M., Marisa, J., & Hidayat, R. (2023). Service per conception on sheep in Langkat District. *Asian Journal of Advances in Agricultural Research*, 21(1), 48-51. https://doi.org/10.9734/ajaar/2023/v21i1410
- Siti, A., & Rachmawati, A. (2024). Reproductive evaluation of beef cattle inseminated with frozen semen of Wagyu cattle in Situbondo District. *International Journal of Current Science Research* and Review, 7(6). https://doi.org/10.47191/ijcsrr/v7i6-100
- Souza, D., Biscarde, C., Machado, W., Vieira, R., Mendes, C., Araújo, M. & Barbosa, L. (2021). Application of hormonal subdoses at the Bai Hui acupoint for estrus synchronization in sheep. *Semina Ciências Agrárias*, 42(4), 2359-2370. https://doi.org/10.5433/1679-0359.2021v42n4p2359

- Şonea, C., Gheorghe-Irimia, R. A., Tapaloaga, D., Gurau, M. R., Udrea, L., & Tapaloaga, P. R. (2023b). Optimizing animal nutrition and sustainability through precision feeding: A mini review of emerging strategies and technologies. *Annals of* "Valahia" University of Târgovişte. Agriculture, 15(2), 7-11.
- Şonea, C., Gheorghe-Irimia, R.A., Tăpăloagă, D., & Tăpăloagă, P.R. (2023a). Nutrition and animal agriculture in the 21st century: A review of future prospects. Annals of the University of Craiova -Agriculture, Montanology, Cadastre Series, 53(1), 303-312
- Wu, D., Wang, C., Simujide, H., Liu, B., Chen, Z., Zhao, P., ... & Chen, A. (2022). Reproductive hormones mediate intestinal microbiota shifts during estrus synchronization in grazing Simmental cows. *Animals*, 12(14), 1751. https://doi.org/10.3390/ani12141751
- Yang, H. (2024). Genome-wide comparative analysis reveals selection signatures for reproduction traits in prolific Suffolk sheep. *Frontiers in Genetics*, 15, 1404031. https://doi.org/10.3389/fgene.2024.1404031
- Zeng, F., Li, C., Huang, J., Xie, S., Zhou, L., Li, M., & Zhang, S. (2021). Glutathione S-transferase kappa 1 is positively related with sperm quality of porcine sperm. *Molecular Reproduction and Development*, 89(2), 104-112. https://doi.org/10.1002/mrd.23551
- Zhang, J., Chenglong, Z., Tuersuntuohe, M., & Liu, S. (2023). Population structure and selective signature of sheep around Tarim Basin. *Frontiers in Ecology and Evolution*, 11. https://doi.org/10.3389/fevo.2023.1146561

161