第 49 卷 第 10 期
2022 年 10 月

湖南大学学报（自然科学版）
Journal of Hunan University（Natural Sciences）

Vol. 49 No. 10
October 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.10.2

Resistance of Simmental Cattle Semen to Time Exposure at Room Temperature

Facroerrozi Hoesni, Silvia Erina, Sri Arnita Abu Tani, Firmansyah*

Faculty of Animal Science, Jambi University, Jambi, Indonesia

Abstract: This research aimed to determine the resistance of Simmental cattle semen with different lifting times at room temperature. It had a completely randomized design (CRD) with seven treatments and five replications. The treatments involved lifting clotted semen at different times from the surface of liquid nitrogen at room temperature: P0 (Control), P1 (straw with lifting time of 5 seconds), P2 (straw with lifting time of 10 seconds), P3 (straw with lifting time of 15 seconds), P4 (straw with lifting time of 20 seconds), P5 (straw with lifting time of 25 seconds), and P6 (straw with lifting time of 30 seconds). Observed variables were motility, percentage of life, and sperm abnormalities. Analysis of variance was used to analyze the data. Duncan's multiple range test determined the significant effect. The results showed that the placement of Simmental cattle clotted semen at room temperature with different lifting times significantly influenced the motility and percentage of sperm life ($P < 0.05$), but it did not impact sperm abnormality significantly ($P > 0.05$). The conclusion is that the placement of Simmental cattle-clotted semen up to 20 seconds under a room temperature (28-30℃) was still feasible and a favorite for artificial insemination. The novelty of this research is to find the right and appropriate time for the placement of frozen semen for Simmental cattle so that the percentage of motility and survival of spermatozoa does not change or the resistance of frozen semen of Simmental cattle is still well maintained, making it easier for inseminators perform livestock insemination in the field conditions.

Keywords: simmental cattle clotted semen, spermatozoa, liquid nitrogen, room temperature, time exposure.

西門塔爾牛精液在室溫下對時間暴露的抵抗力

摘要：本研究旨在測定不同提提時間的西門塔爾牛精液在室溫下的抗性。它採用完全隨機化設計，包含七種處理和五次重複。處理涉及在不同時間從液氮表面在室溫下提起凝固的精液：P0（對照）、P1（提拉時間為 5 秒）、P2（提拉時間為 10 秒）、P3（提拉時間為10秒）提升時間15秒）、P4（吸管提升時間20秒）、P5（吸管提升時間25秒）、P6（吸管提升時間30秒）。觀察到的變量是活力、生命百分比和精子異常。使用方差分析來分析數據。鄧肯的多範圍測試確定了顯著效果。結果表明，西門塔爾牛凝固精液在室溫下放置不同時間，對精子活力和壽命百分率有顯著影響（$P<0.05$），但對精子畸形無顯著影響（$P>0.05$）。結論是，西門塔爾牛凝精液在室溫（28…30℃）下放置20秒仍然是可行的，是人工授精的首選。本研究的新穎之處在於找到合適的西門塔爾牛冷凍精液放置時間，使西門塔爾牛的精子活力和存活率不發生變化，或者西門塔爾牛冷凍精液的抵抗力仍然得到很好的維持，使得授精員在田間條件下更容易為牲畜授精。

关键词：西門塔爾牛凝固精液、精子、液氮、室溫、暴露時間。

Received: July 13, 2022 / Revised: August 11, 2022 / Accepted: September 17, 2022 / Published: October 30, 2022

Fund Project: Faculty of Animal Science, Jambi University (DIPA PNBP funds from the Faculty of Animal Husbandry, applied research scheme for the fiscal year 2021)

About the authors: Facroerrozi Hoesni, Silvia Erina, Sri Arnita Abu Tani, Firmansyah, Faculty of Animal Science, Jambi University, Jambi, Indonesia

Corresponding author Firmansyah, firmansyah_fapet@unja.ac.id

1. Introduction

Simmental cattle is a type of cattle that has been widely developed in Indonesia due to high meat production properties and fast growth. They are very famous in Europe and the original has a red to brown with a white pattern on the head, legs and tail. Simmental cattle are the beef cattle with a body weight gain up to 1–1.5 kg per day. This cow is well-known for breastfeeding well, fast growth, long and dense, including the body weight at birth, wean, and adult.

Artificial Insemination Acceleration program could accelerate population development and improve the genetic quality of livestock. To support the program, superior Simmental cattle breeds can be obtained at the Agriculture Horticultural and Animal Husbandry Services of Jambi Province. Artificial insemination is a process of mating animals artificially with complex procedures that trained officers can apply. This technology has long been applied to enhance of using superior males [1]. The application of this technology is also more efficient than natural mating due to semen or sperm produced by a superior male in one ejaculation can be used to serve more than one female after the semen is processed into frozen semen [2–3].

Longer storage of semen will increase the mortality rate of spermatozoa due to damage to the plasma membrane and result in the disruption of spermatozoa energy supply, thus, could reduce motility, the number of dead spermatozoa. At the end will affect to the spermatozoa that live during the storage process [4–5].

At the dilution process, semen must avoid overheating, direct contact with outside air, and direct sunlight [6–7]. was also during the transferring Semen from a thermos to water or when frozen Semen was transferred from one container to other containers, it must use sterilized tweezers, take times as short as possible and reduce shocks. Adding that damage to spermatozoa by 20% at the time of freezing is still considered satisfactory for inspection [8]. Since the fertility of sperm is still uncertain, when transferring frozen sperm, it is necessary to avoid contact with the human body and to keep it 3-5 seconds outside the container. Artificial inseminators had not found out how long frozen Semen can last with outside air. Based on these circumstances, research was conducted to determine the resistance of Simmental cattle semen with different lifting times at room temperature [9–10].

2. Materials and Methods

This research was carried out in the Integrated Laboratory, University of Jambi. The materials used were 35 straws of clotted Simmental Cattle Semen, collected from the Horticultural and Animal Husbandry Services of Jambi Province. Clotted semen comes from BIB Lembang which were tightly closed and put into liquid N_2. The tools dna equipment used were microscopes, stopwatches, glass objects, tissue, glass cover, containers, scissors, thermometer and clamp.

The experiment was designed into Completely Randomized Design (CRD) with 7 treatments and 5 replications so that there were 35 experimental units. The treatments were:

- P0 - straw without lifting time (control);
- P1 - straw with 5 seconds lifting time;
- P2 - straw with 10 seconds lifting time;
- P3 - straw with 15 seconds lifting time;
- P4 - straw with 20 seconds lifting time;
- P5 - straw with 25 seconds lifting time;
- P6 - straw with 30 seconds lifting time.

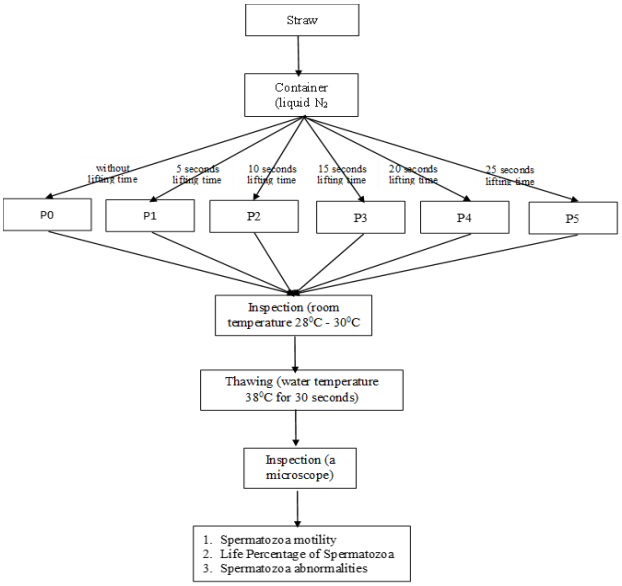

Fig. 1 Research stages

Lifting the straw out from the container by calculating a different time using a Goblet. Each straw was checked at room temperature between 28^0C - 30^0C, then left at a 38^0C temperature for 30 seconds and then viewed under a microscope. Sperm motilities were observed by dripping Semen into a glass object, covered with a glass cover, then observed under a microscope with a magnification of 10 × 40. The estimation of spermatozoa motility by viewing a total of eight fields of view, motility was expressed in (%).

The treatment of cover preparations to calculate the percentage of live sperm must be done in a short time, after being dried it can be directly examined using a microscope. The percentage of lived sperm had no color absorption in spermatozoa. Two drops of dye were placed in a clean, warm glass object at 38^0C temperature. Then, a small drop of Semen was added and mixed and then reviewed using another object glass. The calculation was based on a comparison between the number of lived sperm which was marked with a colorless head and the dead sperm which was marked by colored sperm because eosin could penetrate only on damaged sperm cells. Total sperm observed and expressed in percentage (%).

Percentage of lived sperm, %:

$$\% = \frac{\sum \text{Spermatozoa living}}{\sum \text{spermatozoa accounted}} \times 100\%$$

Deviations in the morphological form of spermatozoa could reduce the fertility of spermatozoa and it was categorized as sperm abnormality. Sperm abnormalities included head and tail abnormality. Head abnormalities were too large, too small, and double head (duplicate head). Tail abnormality was circular tail and double tail. Abnormalities were counted using curing preparations examined under a microscope with a magnification of 100× a minimum of 200 spermatozoa's cells.

Percentage of spermatozoa abnormalities, %:

$$\% = \frac{\text{number of abnormal sperm}}{total\ of\ accounted\ sperm} \times 100\%$$

The data obtained were analyzed using analysis of variance (ANOVA). If the treatment significantly affected the observed variables, the data were further checked using Duncan's multiple range test.

The mathematical model used in this study was

$Yij = \mu + \tau i + \varepsilon ij$

where:

Yij - observation value for i-th treatment and j-th replication;

μ - general average;

i - treatment;

j – replication;

εij - error (gallat) i-th treatment and j-th replication.

3. Results and Discussion

3.1. Spermatozoa Motility

The average percentage of Simmental cattle spermatozoa mortality can be seen in Table 1. Analysis of variance showed that increased duration of lifting time of straw the room temperature, which was 28°C-30°C temperature had a significant effect ($P < 0.05$) on the motility of spermatozoa of Simmental cattle.

The highest motility percentage was found in treatment P0 (59.37%), followed by P1 (58.00%), P2 (56.50%), P3 (54.25%), P4 (44.88%), P5 (35.88%), and P6 (20.50%). The different result might be due to differences in lifting time used, where a decrease in the percentage of spermatozoa motility occurred because when straw was at room temperature, it would evaporate, loss of nitrogen very drastic and decrease their ability to move. In additon, there was also an increase in metabolism, which could increase the lactic acid production, decrease pH and finally create an acidic state, thus, spermatozoa could die.

The loss of liquid nitrogen might cause temperature fluctuations through evaporation during storage, mainly due to high air temperatures, where such conditions causing contact of frozen semen with air temperature not being able to be avoided so the sperm in straw would be in a "shock" due to changes in temperature [11–12]. These conditions could cause the quality of spermatozoa to decrease. During the metabolic process, lactic acid would be produced. If there is no supplied energy to conduct spermatozoa, so, the produced lactic acid could reduce pH both aerobic and anaerobically [13–14]. Spermatozoa have a very short life span especially when spermatozoa were stored at room temperature [4, 11].

Table 1 Average % of Simmental cattle spermatozoa motility

Treatment	Replication					Average (%)
	1	2	3	4	5	
P0	60.00	59.37	58.13	59.37	60.00	59.37 ± 0.77^{f}
P1	58.75	58.13	57.50	57.50	58.13	58.00 ± 0.52^{ef}
P2	57.50	56.88	56.88	56.25	55.00	56.50 ± 0.95^{e}
P3	55.00	55.63	54.38	53.75	52.50	54.25 ± 1.20^{d}
P4	46.25	45.00	45.63	45.00	42.50	44.88 ± 1.43^{c}
P5	37.50	36.88	36.25	35.00	33.75	35.88 ± 1.51^{b}
P6	23.75	22.50	20.00	18.75	17.50	20.50 ± 2.59^{a}

Note: Superscripts with different letters in the same column show significant difference ($P < 0.05$).

For treating P5 (35.88%) and P6 (20.50%), spermatozoa were inappropriate for use because the their motility was < 40% minimum standard. The semen in these treatment groups was not suitable for dissemination and was used in artificial insemination. Evaluation of the motility of post-thawing spermatozoa was one of the many parameters used to determine the quality of cow semen to be used for insemination. The minimum motility of post-thawing spermatozoa was one of the many parameters used to determine the quality of cattle semen that would be used for artificial insemination. The minimum motility of post-thawing spermatozoa was 40% [15–17]. Thawing for 30 seconds resulted better on the motility of live spermatozoa than thawing for 15 seconds [15, 18–19].

3.2. Life Percentage of Spermatozoa

The average life percentage of Simmental cattle spermatozoa can be seen in Table 2. Analysis of variance showed that the length of lifting time at room temperature ranged from 28-30^{0}C significantly affected ($P < 0.05$) the survival percentage of Simmental cattle spermatozoa. The highest percentage of life was in treatment P0 (63.29%) followed by P1 (55.78%) P2 (53.72%) P4 (53.04%) P5 (35.13%) and P6 (21.01%). This condition might be due to the longer lifting time caused in damage to the plasma membrane, which resulted in the disruption of the energy supply, thus decreased spermatozoa life percentage. Died spermatozoa become toxic to other live spermatozoa,

so that in general decreases the quality of spermatozoa decreases [11, 20–21]. The toxic substances from dead spermatozoa or substances containing oxidated diluents during the storage could cause high levels of free radicals and damage the spermatozoa plasma membrane integrity [22, 20]. The function of the plasma membrane was as a protective cell. A damaged membrane may cause the disruption of intracellular metabolic processes, so that spermatozoa may be weak and even die [22].

Table 2 Average % of life of spermatozoa in Simmental cattle

Treatment	Replication					Average %
	1	2	3	4	5	
P0	65.06	63.18	63.28	63.95	60.98	63.29 ± 1.34^{e}
P1	56.73	53.73	56.46	54.76	57.25	55.78 ± 1.33^{d}
P2	53.64	51.30	54.67	52.49	56.52	53.72 ± 1.80^{c}
P3	54.06	52.51	51.97	53.44	53.23	53.04 ± 0.73^{c}
P4	51.39	51.53	52.01	52.81	51.45	51.84 ± 0.53^{c}
P5	35.98	34.84	34.93	37.45	32.45	35.13 ± 1.64^{b}
P6	23.53	21.43	21.19	21.40	17.50	21.01 ± 1.95^{a}

Note: Superscripts with different letters in the same column show significant difference ($P < 0.05$).

Semen in groups P5 (35.13%) and P6 (21.01%) were not suitable for artificial insemination since they were lower than the 50% standard percentage. Good semen is colorless even though the surrounding environment had color; the head had no color (transparent) due to normal membrane permeability and was not damaged. The dead spermatozoa membrane was unable to prevent the ingress of the dye because of damage [23–24, 18]. As a result, the head of the dead spermatozoa is colored like pink eosin. The conditions of spermatozoa easily damaged during treatment and storage can result in the inability to maintain quality [26–27].

3.3. Spermatozoa Abnormalities

The average percentage of Simmental cattle spermatozoa abnormalities can be seen in Table 3. Based on the analysis of variance, the increase in lifting time at room temperature (28-30^{o}C) had no effect on the normality of Simmental cattle semen. The average abnormality was not more than 20%, so semen was appropriate to use for insemination [27]. If the number of abnormal spermatozoa was too high, the degree of spermatozoa fertility decreased, and they could not fertilize ova. The abnormality might occur in the tubuli seminiferi and epididymis. Besides, the disease occurring in the testes might cause the abnormalities [28–29].

Table 3 Average of abnormalilty spermatozoa percentage

Treatment	Replication					Average (%)
	1	2	3	4	5	
P0	6.02	6.20	5.07	5.58	5.69	5.71 ± 0.44
P1	5.09	6.34	5.90	4.56	3.80	5.14 ± 1.02
P2	5.36	6.32	5.75	4.65	5.68	5.55 ± 0.61
P3	6.36	5.79	5.73	6.88	4.94	5.94 ± 0.73
P4	6.59	7.12	5.70	5.78	6.93	6.42 ± 0.65
P5	5.19	5.16	5.14	5.21	4.96	5.13 ± 0.10
P6	3.81	4.08	4.30	3.67	3.99	3.97 ± 0.24

Spermatozoa abnormalities are classified into two groups, namely, primary and secondary abnormalities. Forms of primary spermatozoa abnormalities included a head that was too small or too large, the head was wide or short, elongated, multiplied and shaped like a baby (pyriformis), body or multiple tails [28, 30]. Forms of secondary abnormality include folded tails and proximal or distal cytoplasmic granules and distal disks, and dislocated chromosomes from the head without a tail, and a severed tail [29].

4. Conclusion

Based on the results and discussion of this study, it can be concluded that the placement of Simmental cattle-clotted semen up to 20 seconds at a room temperature of 28-30℃ was still feasible and good for Artificial Insemination. The novelty of this research is to find the right and appropriate time for the placement of frozen semen for Simmental cattle so that the percentage of motility and survival of spermatozoa does not change or the resistance of frozen semen of Simmental cattle is still well maintained, making it easier for inseminators to perform livestock insemination in conditions in the field. The limitation of this research is that this it is only for frozen semen of Simmental cattle, and cannot describe or represent frozen semen of other cattle, especially local cattle such as frozen semen of Bali cattle. In fact, Bali cattle are widely available in field conditions or at the breeder level, which requires frozen semen. For this reason, further research is needed for frozen semen of local cattle.

Acknowledgement

Acknowledgments are conveyed to the Faculty of Animal Science, Jambi University, which has funded this research through DIPA PNBP funds from the Faculty of Animal Husbandry, applied research scheme for the fiscal year 2021.

References

[1] AGUSTINE, R., BINTARA, S., ANDARWATI, S., MUZAYYANAH, M.A.U., WIDI, T.S.M., and PUTRA, A.R.S. Analysis in Making Decision of Farmer to Select Bull Frozen Semen in Indonesia. *Journal of the Indonesian Tropical Animal Agriculture*, 2019, 44(3): 323-332. https://doi.org/10.14710/jitaa.44.3.323-332

[2] LIAMAS-LUCENO N., HOSTENS M., MULLAART E., BROEKHUIJSE M., LONERGAN P., and SOOM A.V. High Temperature-Humidity Index Compromises Sperm Quality and Fertility of Holstein Bulls In Temperate Climates. *Journal of Dairy Science*, 2020, 103(10): 9502-9514. https://doi.org/10.3168/jds.2019-18089

[3] AZURA S. RATNANI H., SOEPRANIANONDO K., SUSILOWATI S., HARIADI M., and SAMIK A. Effect of α-Tocopherol Supplementation in Diluent on the Motility, Viability and Plasma Membrane Integrity of Simmental Bull Spermatozoa after Cooling. *OVOZOA: Journal of Animal Reproduction*, 2020, 9(1): 1-6. http://dx.doi.org/10.20473/ovz.v9i1.2020.1-6

[4] ALOMAR M., ZARKAWI M., and ALZOABI M.A. Analysis of Awassi Sperm Motility in Two Media at Different Levels of Temperatur, pH and Osmolality. *Iranian Journal of Applied Animal Science*, 2018, 8(3): 431-438. https://ijas.rasht.iau.ir/article_542635_c0bcf1876874c8fb668715be13526185.pdf

[5] AHMAD S.B., AHMAD I., AHMAD N., QURESHI Z.I., JAMIL H., ALI Q., and ASHFAQ K. Effect of Addition of Different Concentrations of Alpha Lipoic Acid to Tris Egg Yolk Citrate Glycerol Extender on Cryopreservation of Sahiwal Bull Spermatozoa. *Pakistan Veterinary Journal*, 2018, 38(3): 301-305. http://dx.doi.org/10.29261/pakvetj/2018.060

[6] ZAIDI N.S., and ANWAR M. Effect of Biostimulation on Estrus Expression Resumption on Ovarian Activity and Concedption Rate in Postpartum Anestrus Nili-Ravi Buffaloes during Low Breeding Season. *Pakistan Veterinary Journal*, 2018, 38(1): 35-38. DOI:10.29261/pakvetj/2018.007

[7] SHARMA M., YAQOOB B., SINGH A., SHARMA N., and RAWAT S. Effect of Temperature Humidity Index on Semen Quality of Bovine Bull. *International Journal of Current Microbiology and Applied Sciences*, 2017, 6(12): 1822-1830. https://doi.org/10.20546/ijcmas.2017.612.206

[8] SABES-ALSINA M., LUNDEHEIM N., JOHANNISSON A., LOPEZ-BEJAR M., and MORRELL J.M. Relationships Between Climate and Sperm Quality in Dairy Bull Semen: A retrospective analysis. *Journal of Dairy Science*, 2019, 102(6): 5623-5633. https://doi.org/10.3168/jds.2018-15837.

[9] MURPHY E.M., KELLY A.K., O'MEARA C., EIVERS B., LONERGAN P., and FAIR S. Influence of Bull Age, Ejaculate Number, and Season of Collection on Semen Production and Sperm Motility Parameters in Holstein Freisian Bulls in a Commercial Articial Insemination Centre. *Journal of Animal Science*, 2018, 96(6): 2408-2418. https//doi.org/10.1093/jas/sky.130.

[10] PACHECO E.B.P., ADEGBEYE M.J., LAGUNAS B.C., SALAS J.M., AGUILAR A.S., and HEVERASTICO C.C. Effect of Artificial Insemination and Natural Mating on Reproductive Parameters in Pigs of Warm-Humid Climate Region. *Indian Journal of Animal Science*, 2020, 90(3): 372-374. https://doi.org/10.56093/ijans.v90i3.102425

[11] MURPHY E.M., MURPHY C., O'MEARA C., DUNNE G., EIVERS B., LONERGAN P., and FAIR S. A Comparison of Semen Diluents on the in Vitro ang in Vivo Fertility of Liquid Bull Semen. *Journal of Dairy Science*, 2017, 100(2): 1541-1554. https://doi.org/10.3168/jds.2016-11646

[12] SUPRAYOGI T.W. and SUSILOWATI S. The Effect of Cattle Seminal Plasma Crude Protein on the Cryopreservation of Goat Semen. *Iranian Journal of Applied Animal Sciences*, 2018, 8(4): 641-646. https://ijas.rasht.iau.ir/article_544772_c79855acde0663156b20b537845696d4.pdf

[13] KHALIL W.A., EL-HARAIRY M.A., ZEIDAN A.E.B., HASAN M.A.E., and MOHEY-ELSAEED O. Evalution of Bull Spermatozoa During and after Cryopreservation Structural and Ultrastructural Insights. *International Journal of Veterinary Science and Medicine*, 2018, 6(Suppl): 49-56. https://doi.org/10.1016/j.ijvsm.2017.11.001

[14] DRAKE E., HOLDEN S.A., AUBET V., DOYLE R.C., MILLAR C., MOORE S.G. MAICAS C., RANDI F. CROMIE A.R., LONERGAN P., and BUTLER S.T. Evaluation of Delayed Timing of Aritficial Insemination with Sex-Sorted Sperm on Pregnancy per Aritificial Insemination in Seasonal-Calving Pasture-based Lactating Dairy Cow. *Journal of Dairy Science*, 2020, 103(12): 12059-12068. https://doi.org/10.3168/jds.2020-18847

[15] MEMILI E., MOURA A.A., and KAYA A. Metabolisme of Sperm and Seminal Plasma Associated with Bull Fetility. *Animal Reproduction Science*, 2020, 220: 106355. DOI: 10.1016/j.anireprosci.2020.106355

[16] AL-BADRY K.I., ABOUD Q.M., ZALZALA S.J., IBRAHIM F.F., and LATEEF W.Y. Effect of Trehalose and Month of Collection on DNA Fragmentation in Holstein Bull Semen during Dilution, Cooling and Thawing. *Online Journal of Veterinary Research*, 2018, 22(3): 237-246.

[17] ZARGARAN, A., AFSHAR, M.A., JOEZY-SHEKALGORABI S., AZIZI J., and M. CHAMANI M. Reproductive Performance of Holstein Heifers Inseminated With Sex Sorted Semen in Various Herd Sizes. *Iranian Journal of Applied Animal Sciences*, 2021, 11(2): 249-259. https://ijas.rasht.iau.ir/article_682242_b562ceea81dfb7f2338920914df76c58.pdf

[18] SUHARDI R., MEGAWATI N., ARDHANI F., SUMMPUNN P., and WUTHISUTHIMETHAVEL S. Motility, Viability, and Abnormality of the Spermatozoa of Bali Bull with Andromed and Egg Yolk, Tris Diluents Stored at 4 degrees C. *Iranian Journal of Applied Animal Science*, 2020, 10(2): 249-256. https://ijas.rasht.iau.ir/article_673200_4988ec756516b6361f055f9a8311e47a.pdf

[19] KAKA A., HARON W., YUSOFF R., YIMER N., KHUMRAN A.M., MEMON A.A., SARSAIFI K., and EBRAHIMI M. Frosen-Thawed Quality of Bull Semen after Combind Suppplementation of Docosahexaenoic Acid and Alpha Linolenic into Tris based Semen Extender. *Pakistan Journal of Zoology*, 2019, 49(6): 2051-2055. DOI:10.17582/journal.pjz/2017.49.6.2051.2055

[20] MAHDI, S.W., AL-SHAMARY, S.M., and JAAFIR, J.S. Role of Spermatozoa in pH Stability of Caudal Epididymis Enviroment. *Iraqi Journal of Veterinary Sciences*, 2019, 33(1): 111-116. https://doi.org/10.33899/ijvs.2019.125511.1033
[21] KHAN I.M., KHAN R.U., QURESHI M.S., USMAN T., KHAN A., ULLAH Z., and REHMAN H. Cross Breeding Promotes Deterioration of Semen Quality in Cattle Bulls. *Pakistan Journal of Zoology*, 2018, 50(1): 97-103. http://dx.doi.org/10.17582/journal.pjz/2018.50.1.97.103
[22] NAZARI M., KIA H.D., and NAJAFI A. Effect of Pentoxifylline Antioxidant Suplementation on Improvement of Sperm Motility Parameters in Non-Breeding Season. *Iranian Journal of Animal Science Research*, 2022, 14(1): 55-64. https://doi.org/10.22067/ijasr.2021.38267.0
[23] MAICAS C., HUTCHINSON I.A., KENNEALLY J., GRANT J., CROMIE A.R., LONERGAN P., and BUTLER S.T. Fertility of Fresh and Frozen Sex-Sorted Semen in Dairy Cows and Heifers in Seasional-Calving Pasture-Based Herds. *Journal of Dairy Science*, 2019, 102(11): 10530-10542. https://doi.org/10.3168/jds2019-16740.
[24] YENDRALIZA T., MISRIANTI R., and ZURMARNI. Viability and Recovery Rate of Bali Cattle Spermatozoa during Preservation in Tris-Based Egg Yolk Diluent with Different Sucrose Levels. *Journal of Veterinary Medicine*, 2019, 13(2): 55-60. https://doi.org/10.21157/j.ked.hewan.v13i2.13033
[25] NAGATA M.P.B., EGASHIRA J., KATAFUCHI N., ENDO K., OGATA K., YAMANAKA K., YAMANOUCHI T., MATSUDA H., HASHIYADA Y., and YAMASHITA K. Bovine Sperm Selection Procedure Prior to Cryopreservation for Improvement os Post-Thawed Semen Quality and Fertility. *Journal of Animal Science and Biotechnology*, 2019, 10: 91. https://doi.org/10.1186/s40104-019-0395-9.
[26] ARIF A.A., MAULANA T., KAIIN E.M., PURWANTARA B., and ARIFIANTINI R. I. The Quality of Frozen Semen of Limousin Bull in Various Semen Diluents. *Tropical Animal Science Journal*, 2022, 45(3): 284-290. https://doi.org/10.5398/tasj.2022.45.3.284
[27] MACEDO S., BLIEBERNICHT M., CARVALHEIRA J., COSTA A., RIBEIRO F., and ROCHA A. Effect of Two Freezing Methods and Two Cryopreservation Media on Post-Thaw Quality of Stallion Spermatozoa. *Reproduction in Domestic Animals*, 2018, 53(2): 519-524. https://doi.org/10.1111/rda.13140
[28] SANTOSO H., ARIFIANTINI R.I., GUNAWAN A., and SUMANTRI C. Characteristics and Potential Production of Frozen Semen of Pasundan Bull. *Trofical Animal Science Journal*, 2021, 44(1): 24-31. https://doi.org/10.5398/tasj.2021.44.1.24
[29] SOUTO P.L.G., BARBOSA E.B., DA SILVA F.I.C., MARTINS V.M.V., HATAMOTO-ZERVOUDALIS L.K., MEMANUS C., DE ALENCAR E.R., and RAMOS A.F. Seasonal Semen Quality of a Local and Commercial Taurine Cattle Breeds, Raised in a Subtropical Climate Relationship between External Morphology of the Animals and Climate. *Animal Reproduction Science*, 2022, 240: 106974. https://doi.org/10.1016/j.anireprosci.2022.106974
[30] SAPUTRA D.J., IHSAN M.N., and ISNAINI N. Correlation Between Scrotal Circumference with Semen Volume, Concentration and Spermatozoa Motility of Bali Cow Males. *Tropical Livestock Journal*, 2017, 18(2): 47-53. DOI: 10.21776/ub.jtapro.2017.018.02.9

参考文:

[1] AGUSTINE, R.、BINTARA, S.、ANDARWATI, S.、MUZAYYANAH, M.A.U.、WIDI, T.S.M. 和 PUTRA, A.R.S.
印尼農戶選擇公牛冷凍精液决策分析。印度尼西亞熱帶動物農業雜誌, 2019, 44(3): 323-332. https://doi.org/10.14710/jitaa.44.3.323-332
[2] LIAMAS-LUCENO N.、HOSTENS M.、MULLAART E.、BROEKHUIJSE M.、LONERGAN P. 和 SOOM A.V.高溫濕度指數會影響溫帶氣候下荷斯坦公牛的精子質量和生育能力。乳品科學雜誌, 2020, 103(10): 9502-9514. https://doi.org/10.3168/jds.2019-18089
[3] AZURA S. RATNANI H.、SOEPRANIANONDO K.、SUSILOWATI S.、HARIADI M. 和 SAMIK A. 在稀釋劑中添加α生育酚對冷卻後西門塔爾公牛精子的運動性、活力和質膜完整性的影響。動物繁殖雜誌，2020，9(1)：1-6。http://dx.doi.org/10.20473/ovz.v9i1.2020.1-6
[4] ALOMAR M.、ZARKAWI M. 和 ALZOABI M.A. 在不同溫度、酸鹼度值和滲透壓水平下兩種介質中我建議精子活力的分析。伊朗應用動物科學雜誌，2018年，8(3)：431-438。https://ijas.rasht.iau.ir/article_542635_c0bcf1876874c8fb668715be13526185.pdf
[5] AHMAD S.B.、AHMAD I.、AHMAD N.、QURESHI Z.I.、JAMIL H.、ALI Q. 和 ASHFAQ K. 在三聚體蛋黃檸檬酸甘油增量劑中添加不同濃度的硫辛酸對萨希瓦尔低溫保存的影響公牛精子。巴基斯坦獸醫雜誌, 2018, 38(3): 301-305。http://dx.doi.org/10.29261/pakvetj/2018.060
[6] ZAIDI N.S. 和 ANWAR M. 生物刺激對發情期表達恢復對產後低發情期尼羅-拉維水牛卵巢活性和受精率的影響。巴基斯坦獸醫雜誌, 2018, 38(1): 35-38. DOI:10.29261/pakvetj/2018.007
[7] SHARMA M.、YAQOOB B.、SINGH A.、SHARMA N. 和 RAWAT S. 溫濕度指數對公牛精液質量的影響。國際當代微生物學與應用科學雜誌, 2017, 6(12): 1822-1830。https://doi.org/10.20546/ijcmas.2017.612.206
[8] SABES-ALSINA M.、LUNDEHEIM N.、JOHANNISSON A.、LOPEZ-BEJAR M. 和 MORRELL J.M. 氣候與奶牛精液中精子質量的關係：回顧性分析。乳業科學雜誌, 2019, 102(6): 5623-5633.

https://doi.org/10.3168/jds.2018-15837。

[9] MURPHY E.M.、KELLY A.K.、O'MEARA C.、EIVERS B.、LONERGAN P. 和 FAIR S. 公牛日齡、射精次數和採集季節對荷斯坦弗萊西安公牛精液產量和精子活力參數的影響在商業人工授精中心。動物科學雜誌, 2018, 96(6): 2408-2418。https//doi.org/10.1093/jas/sky.130。

[10] PACHECO E.B.P.、ADEGBEYE M.J.、LAGUNAS B.C.、SALAS J.M.、AGUILAR A.S. 和 HEVERASTICO C.C. 暖濕氣候區人工授精和自然交配對豬繁殖參數的影響。印度動物科學雜誌，2020年，90(3)：372-374。https://doi.org/10.56093/ijans.v90i3.102425

[11] MURPHY E.M.、MURPHY C.、O'MEARA C.、DUNNE G.、EIVERS B.、LONERGAN P. 和 FAIR S. 精液稀釋劑對液體公牛精液體外受精能力的比較。乳品科學雜誌, 2017, 100(2): 1541-1554。https://doi.org/10.3168/jds.2016-11646

[12] SUPRAYOGI T.W. 和 SUSILOWATI S. 牛精漿粗蛋白對山羊精液冷凍保存的影響。伊朗應用動物科學雜誌，2018年，8(4)：641-646。https://ijas.rasht.iau.ir/article_544772_c79855acde0663156b20b537845696d4.pdf

[13] KHALIL W.A.、EL-HARAIRY M.A.、ZEIDAN A.E.B.、HASAN M.A.E. 和 MOHEY-ELSAEED O. 公牛精子在低溫保存期間和之後的結構和超微結構洞察評估。國際獸醫科學與醫學雜誌, 2018, 6(補充): 49-56. https://doi.org/10.1016/j.ijvsm.2017.11.001

[14] DRAKE E.、HOLDEN S.A.、AUBET V.、DOYLE R.C.、MILLAR C.、MOORE S.G. MAICAS C.、RANDI F. CROMIE A.R.、LONERGAN P. 和 BUTLER S.T. 季節性產犢牧場泌乳奶牛人工授精性別分選精子人工授精延遲時間評價。乳業科學雜誌, 2020, 103(12): 12059-12068。https://doi.org/10.3168/jds.2020-18847

[15] MEMILI E.、MOURA A.A. 和 KAYA A. 與公牛生育能力相關的精子和精漿代謝。動物生殖科學, 2020, 220: 106355。DOI:10.1016/j.anireprosci.2020.106355

[16] AL-BADRY K.I.、ABOUD Q.M.、ZALZALA S.J.、IBRAHIM F.F. 和 LATEEF W.Y. 海藻糖和採集月份對稀釋、冷卻和解凍過程中荷斯坦公牛精液中脫氧核糖核酸片段化的影響。在線獸醫研究雜誌，2018年，22(3)：237-246。

[17] ZARGARAN, A., AFSHAR, M.A., JOEZY-SHEKALGORABI S., AZIZI J., 和 M. CHAMANI M. 荷斯坦小母牛在不同畜群規模中用性別分選精液授精的繁殖性能。伊朗應用動物科學雜誌，2021，11(2)：249-259。https://ijas.rasht.iau.ir/article_682242_b562ceea81dfb7f2338920914df76c58.pdf

[18] SUHARDI R., MEGAWATI N., ARDHANI F., SUMMPUNN P. 和 WUTHISUTHIMETHAVEL S. 巴厘岛公牛精子的运动性、活力和异常，仙女座和蛋黄，三聚体稀释剂储存在4摄氏度。伊朗應用動物科學雜誌，2020，10(2)：249-256。https://ijas.rasht.iau.ir/article_673200_4988ec756516b6361f055f9a8311e47a.pdf

[19] KAKA A.、HARON W.、YUSOFF R.、YIMER N.、KHUMRAN A.M.、MEMON A.A.、SARSAIFI K. 和 EBRAHIMI M. 冰凍的-將二十二碳六烯酸和α-亞麻酸聯合補充後公牛精液的解凍質量基於三聚體的精液增量劑。巴基斯坦動物學雜誌, 2019, 49(6): 2051-2055。DOI:10.17582/journal.pjz/2017.49.6.2051.2055

[20] MAHDI, S.W.、AL-SHAMARY, S.M. 和 JAAFIR, J.S. 精子在附睾尾部環境酸鹼度穩定性中的作用。伊拉克獸醫學雜誌, 2019, 33(1): 111-116. https://doi.org/10.33899/ijvs.2019.125511.1033

[21] KHAN I.M.、KHAN R.U.、QURESHI M.S.、USMAN T.、KHAN A.、ULLAH Z. 和 REHMAN H. 雜交育種促進公牛精液質量惡化。巴基斯坦動物學雜誌, 2018, 50(1): 97-103. http://dx.doi.org/10.17582/journal.pjz/2018.50.1.97.103

[22] NAZARI M.、KIA H.D. 和 NAJAFI A. 補充己酮可可鹼抗氧化劑對非繁殖季節精子活力參數改善的影響。伊朗動物科學研究雜誌, 2022, 14(1): 55-64。https://doi.org/10.22067/ijasr.2021.38267.0

[23] MAICAS C.、HUTCHINSON I.A.、KENNEALLY J.、GRANT J.、CROMIE A.R.、LONERGAN P. 和 BUTLER S.T. 季節性產犢牧場牛群中奶牛和小母牛的新鮮和冷凍性別分選精液的生育力。乳業科學雜誌, 2019, 102(11): 10530-10542。https://doi.org/10.3168/jds2019-16740。

[24] YENDRALIZA T.、MISRIANTI R. 和 ZURMARNI。巴厘島牛精子在不同蔗糖水平的 三聚體基蛋黃稀釋液中保存期間的活力和回收率。獸醫學雜誌, 2019, 13(2): 55-60。https://doi.org/10.21157/j.ked.hewan.v13i2.13033

[25] NAGATA M.P.B.、EGASHIRA J.、KATAFUCHI N.、ENDO K.、OGATA K.、YAMANAKA K.、

YAMANOUCHI T.、MATSUDA H.、HASHIYADA Y. 和 YAMASHITA K. 牛精子冷凍保存前的選擇程序改善解凍後的精液質量和生育力。動物科學與生物技術雜誌，2019年，10：91。https://doi.org/10.1186/s40104-019-0395-9。

[26] ARIF A.A.、MAULANA T.、KAIIN E.M.、PURWANTARA B. 和 ARIFIANTINI R.I. 各種精液稀釋劑中利木贊公牛冷凍精液的質量。熱帶動物科學雜誌, 2022, 45(3): 284-290。https://doi.org/10.5398/tasj.2022.45.3.284

[27] MACEDO S., BLIEBERNICHT M., CARVALHEIRA J., COSTA A., RIBEIRO F., 和 ROCHA A. 兩種冷凍方法和兩種冷凍保存介質對種馬精子解凍後質量的影響。家畜繁殖, 2018, 53(2): 519-524。https://doi.org/10.1111/rda.13140

[28] SANTOSO H.、ARIFIANTINI R.I.、GUNAWAN A. 和 SUMANTRI C. 百順丹公牛冷凍精液的特性和潛在產量。熱帶動物科學雜誌, 2021, 44(1): 24-31。https://doi.org/10.5398/tasj.2021.44.1.24

[29] SOUTO P.L.G.、BARBOSA E.B.、DA SILVA F.I.C.、 MARTINS V.M.V.、HATAMOTO-ZERVOUDALIS L.K.、MEMANUS C.、DE ALENCAR E.R. 和 RAMOS A.F. 在亞熱帶氣候關係中飼養的本地和商業牛磺酸牛品種的季節性精液質量動物外部形態與氣候之間的關係。動物生殖科學，2022年，240：106974。https://doi.org/10.1016/j.anireprosci.2022.106974

[30] SAPUTRA D.J.、IHSAN M.N. 和 ISNAINI N. 巴厘島公牛陰囊周長與精液量、濃度和精子活力的相關性。熱帶畜牧雜誌, 2017, 18(2): 47-53。 DOI: 10.21776/ub.jtapro.2017.018.02.9