Animal Science Papers and Reports vol. 43 (2025) no. 3, 273-282
DOI: 10.2478/aspr-2025-0019
Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

Seasonal variation of sex hormones and semen quality in Simental bulls

Alicja Kowalczyk[1], Joanna Gruszczyńska[2]*, Marian Kuczaj[3], Ewa Czerniawska-Piątkowska[4], Beata Grzegrzółka[2], Anna Szul[5], Leszek Hebda[5], Wiesław Piotr Świderek[2]

[1] Wrocław University of Environmental and Life Sciences, Department of Environment Animal Hygiene and Welfare, Chełmońskiego 38C, 51-630 Wroclaw, Poland

[2] Warsaw University of Life Sciences – SGGW, Department of Animal Genetics and Conservation, Ciszewskiego 8, 02-786 Warsaw, Poland

[3] Wrocław University of Environmental and Life Sciences, Faculty of Biology and Animal Science, Chełmońskiego 38C, 51-630 Wroclaw, Poland

[4] West Pomeranian University of Technology in Szczecin, Department of Ruminant Animal Sciences, Klemensa Janickiego 29, 71-270 Szczecin, Poland

5 Malopolska Biotechnic Center Ltd, Krasne 32, 36-007 Krasne, Poland

(Accepted February 28, 2025)

The purpose of this study was to find out whether the seasonal climate changes have any impact on sex hormones levels and semen characteristics in Simmental bulls kept in the insemination station. The study was conducted for a year. Ejaculates of twelve bulls were collected from each bull three times a month using an artificial vagina. Basic semen characteristics - ejaculate volume, sperm concentration, motility, and percentage of live sperm - were assessed in the collected samples. Blood was drawn monthly from the bulls to measure testosterone and estradiol levels. Significant differences were found in the levels of sex hormones in blood serum and in the bull sperm depending on the season. The highest testosterone levels were observed in summer, while estradiol levels in

*Corresponding author: joanna_gruszczynska@sggw.edu.pl

spring. Semen concentration was best in summer, motility in spring and viability in autumn. There was no clear relationship between hormone levels and semen parameters. The obtained results suggest that season has a large impact on the serum testosterone and estradiol levels and also on bovine sperm parameters.

KEY WORDS: cattle / estradiol / testosterone / semen parameters

Ruminants have a higher degree of heat tolerance than monogastric animal species. However, there are also differences among members of the Bovidae family as *Bos indicus* is generally more heat resistant than *Bos taurus* [Burns *et al.* 1997]. Also, with the increase in the milk yield of dairy cattle and the growth rate of beef cattle, metabolic heat production increased, and the ability to tolerate elevated temperatures decreased [Zumbach *et al.* 2008, Dikmen and Hansen 2009]. Finaly, the seasonal variation in climatic conditions is an important factor influencing bull sperm parameters. [Rekwot *et al.* 1987, Goswami *et al.* 1991, Singh and Raina 2000].

Hormones play a significant role in shaping the fertility of bulls, while hormonal activity is affected by the environment. Testosterone and estradiol regulate the spermatogenesis process and determine the quality of the reproductive cells [Godfey *et al.* 1985, Laudat *et al.* 1998, Souza *et al.* 2011, Chacur *et al.* 2013, Al-Delemi and Kadim 2015, Shatab *et al.* 2016]. The activity of these hormones, and thus the quality of sperm, is influenced by genetic and environmental factors (climate, season, housing conditions, nutrition, etc.) Earlier research [Bhakat *et al.* 2014], found that high temperatures, which are especially hard to bear in summer, have an adverse multidimensional effect on reproductive traits, feed consumption and metabolism.

Due to high temperatures, bulls are physically exhausted which significantly affects their sperm quality [Mandal *et al.* 2000]. Most studies have found a significant effect of the season on the parameters of bull semen quality [Rekwot *et al.* 1987, Goswami *et al.* 1991, Singh and Raina 2000, Javed *et al.* 2000a, Farooq *et al.* 2013, Bhakat *et al.* 2014, Nitika *et al.* 2020, Perumal *et al.* 2017], only in a few cases the effect of the season turned out to be insignificant [Mathur *et al.* 2002, Helbig *et al.* 2007].

The ability to thermoregulate depends on a complex interaction between anatomical and physiological factors. Ravagnolo and Misztal [2002] and Bohmanova *et al.* [2005, 2007, 2008] found that balancing adaptation to high temperatures with high production potential is challenging due to distinct physiological and metabolic processes governing heat tolerance, milk yield, and reproductive performance. In beef cattle, the genetic antagonism between adaptation to a high temperature environment and high production potential appears to be more limited than in the case of dairy cattle. In order to improve the adaptive characteristics of animals, reproductive technologies, strategic crossbreeding (crossing commercial breeds with the native local breeds) and assessing animals by molecular markers are employed [Prayaga *et al.* 2006]. It is estimated that the direct impact of climate change on animals is likely to be small as long as the temperature increase does not exceed 3°C [Hoffmann 2010].

The results of studies conducted on Holstein Friesian bulls suggest that breeding programs should incorporate thermal tolerance features and larger consideration of

genotype by environment interaction to identify animals best adapted to specific conditions [Hoffmann 2010, Al-Kanaan *et al.* 2015]. Despite so many studies, there is still no unequivocal analysis of the relationship between climatic and seasonal factors and the level of sex hormones and sperm quality. Moreover, most of the scientific research on the effect of seasons on semen characteristics and sex hormone levels has been carried out using native *Bos indicus* and *Buffalo* bulls in tropical climates. Therefore, the aim of this study was to investigate these relationships in Simmental bulls kept in temperature climate typical of Poland.

Material and methods

This study did not require the consent of the Local Ethical Committee, all animal procedures followed routine practices in compliance with the insemination station's regulations The research was conducted across one year on 12 Simmental bulls (±3 years old) at a single insemination station. (Krasne, Małopolska, Poland, DD: 50.04133, 22.08103, 228). The research material was ejaculate and blood. The animals were healthy, kept in the same environmental and nutritional conditions (indoor temperatures and humidity were not controlled).

Semen was collected from each bull three times a month (in the first week, in the middle of the month, in the fourth week) at a fixed time (7:00 a.m.) using an artificial vagina. Immediately after collection, the semen was transferred to graduated tubes and placed in a water bath at 37°C until its evaluation when its volume was measured using a graduated tube [Kowalczyk *et al.* 2020]. A digital photometer (560 nm) (Dr. Lange, LP 300 SDM; Minitube, Tiefenbach b. Landshut, Germany) was used to assess sperm concentration. Sperm motility was assessed following the procedure described by Mizera *et al.* [2019a] using Computer Assisted Sperm Analyzer (SCA, Microptic, Spain). A flow cytometer (CytoFlex Beckman Coulter, B3-R1-V0, China) was used to analyse spermatozoa viability according to the procedure described by Mizera *et al.* [2019b].

For research purposes, venous blood (5 ml) was taken from the same bulls in the middle of each month (7:00 a.m.). The material was collected into haematological tubes containing lithium heparin at a concentration of 20 IU/ml of blood. Serum samples were stored in a refrigerator at 4°C until evaluation (maximum 4 h). Sex hormone concentrations were assessed using radioimmunoassay reagents to quantify testosterone (TESTO-RIA-CT (KIP1709, DIA Source, Belgium) and estradiol (E2-RIA-CT (KIP0629, DIA Source, Belgium) according to the manufacturer's procedure (https://www.diasource-diagnostics.com/). Climatological data (average values of temperature, humidity and day length) in the analyzed seasons (autumn, winter, spring, summer) were obtained from the Rzeszów-Jesionka meteorological station (50 ° 06'41N, 22 ° 01'12E), https: // pl .meteopost.com / weather / rzeszow-jasionka (Tab. 1).

The obtained data (semen parameters and hormone levels) were subjected to statistical analysis. Both the original data of variables and the transformed ones did not meet the requirements of normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Therefore, nonparametric methods were used.

To assess the differences in semen parameters and hormone concentrations between the bulls in the season (autumn, winter, spring, summer) and between seasons, Friedman's test for dependent samples was applied. Significance values were corrected using the Bonferroni method. In the vast majority of cases, statistical analyses of semen parameters variability in individual seasons of the year did not show significant differences between the bulls. Only single cases of significant relationships were noted in winter (sperm concentration) and autumn (live sperm). Therefore, the statistical study focused on the analysis of seasonal changes in semen parameters and sex hormone concentration. The relationship between estradiol and testosterone and between hormones and semen parameters was assessed using Spearman rank correlation. All statistical calculations were performed using IBM SPSS Statistics 29 [Darren and Paul, 2024].

Results and discussion

Table 1 shows the average seasonal values of temperature, humidity and daylight measurements in the analysed seasons. The lowest average daily temperature (-1.17°C) and the shortest day (8.7 h) were recorded in winter, the highest temperature (19.63°C) and the longest day (15.9 h) in summer. These seasons also differed in air humidity (82.3-71.6%). However, there were no significant differences between spring and autumn.

Our studies showed a significant relationship ($p<0.01$) between the season and estradiol and testosterone levels in the blood serum (Tab. 1). The highest level of

Table 1. Serum estradiol and testosterone levels depending on season and the correlation between these hormones

Season t (°C)/ h (%)/ dl (h)	n	Estradiol (pg/ml)		Testosterone (ng/dl)		r	p-value
		mean	CV %	mean	CV %		
Autumn /9.83/83.6/10.7	36	7.5^{A}	50.7	46.9^{A}	67.0	0.089	0.605
Winter /-1.17/82.3/8.7	36	4.5^{B}	37.8	34.4^{BC}	62.8	0.074	0.667
Spring /9.47/75.3/13.8	36	10.0^{BC}	31.0	96.9^{B}	50.4	-0.219	0.198
Summer /19.63/71.6/15.9	36	3.9^{AC}	21.8	137.6^{AC}	37.2	0.032	0.848
Total	144	6.5	61.5	79.1	87.2	-0.025	0.761

t – temperature. h – humidity. dl – day length. CV – coefficient of variation.
^{ABC}Within columns means bearing the same superscript differ significantly at $p<0.01$.

estradiol was recorded in autumn (7.5 pg/ml) and spring (10.0 pg/ml), significantly the lowest in summer 3.9 pg/ml. In the case of testosterone, the lowest level of this hormone in bulls was observed in autumn-winter (46.9 ng/dl, 34.4 ng/dl, respectively), while the highest in spring (96.9 ng/dl) and summer (137.6 ng/dl). It is worth noting a very high variability of the tested hormones, especially in autumn and winter (estradiol CV = 50.7-37.8%, testosterone CV = 67.0-62.8%) compared to the summer season (estradiol CV = 21.8%, testosterone CV = 37.2%). The estimated correlations between the levels of these hormones in summer, autumn and winter periods were positive (r = 0.032, 0.089, 0.074, respectively), and negative in spring (r = -0.219).

Variations in semen parameters were also observed over the seasons (Tab. 2). The highest volume of ejaculate (6.0-6.2 ml) with the lowest sperm concentration (1.3-1.7×10^6/µl) was obtained from bulls in winter and autumn, while in summer the opposite relationship was found (5.1 ml, 1.8×10^6/µl). Sperm from semen collected in spring showed significantly greater individual motility (77.1%) than in summer (73.8%) and autumn (71.8%). The highest percentage of live sperm in bull semen was recorded in autumn (79.0%), while it was significantly lower in winter (74.9%).

Table 2. Bull semen characteristics across seasons

Season	Ejaculate volume (ml)		Sperm concentration (×10^6/µl)		Individual sperm motility (%)		Live sperm (%)	
	mean	CV %	mean	CV %	mean	CV %	mean	CV %
Autumn (n=108)	6.2^{A}	6.5	1.7^{A}	17.6	71.8^{AB}	1.1	79.0^{Abc}	2.5
Winter (n=108)	6.0	11.7	1.3^{AB}	15.4	76.3^{A}	2.8	74.9^{A}	1.5
Spring (n=108)	5.8	12.1	1.7	29.4	77.1^{Bc}	2.2	76.5^{c}	4.4
Summer (n=108)	5.1^{A}	21.6	1.8^{B}	27.8	73.8^{c}	3.3	76.6^{b}	4.0
Total (n=432)	5.6	30.4	1.6	37.5	74.8	6.1	76.7	7.8

CV – coefficient of variation.
$^{aA...}$Within columns means bearing the same superscript differ significantly at: small letters – $p<0.05$; capitals – $p<0.001$.

The analysed bull semen parameters showed different correlation (Tab. 3). There was a negative correlation between ejaculate volume and sperm concentration (r = -0.287), individual motility (r = -0.072) and the percentage of live sperm (r = -0.101). On the other hand, there was a positive correlation between sperm concentration and sperm motility (r = 0.132) and with the percentage of live sperm (r = 0.119). A positive correlation was also observed between the individual sperm motility and the percentage of live sperm (r = 0.084). The concentrations of testosterone and estradiol in the blood serum of the bulls across different seasons exhibited weak (statistically insignificant) positive (r = 0.003; 0.261) or negative (r = -0.013; -0.329) correlations with semen parameters (Tab. 4). During the autumn season, testosterone showed the highest positive correlation with sperm motility (r = 0.261) and the highest negative correlation with the percentage of live spermatozoa (r = -0.209). In this season, all correlations between estradiol and semen parameters

were positive, with the strongest associations observed for sperm concentration (r = 0.198) and sperm motility (r = 0.224). During winter, there was a considerable variation in the correlation coefficients between hormones and semen quality. For testosterone, low positive correlations predominated, whereas for estradiol, negative correlations were more frequently recorded, with the strongest correlation observed for sperm concentration (r = -0.286). Overall, in spring and summer, negative correlations between sex hormones and semen parameters were observed. For testosterone, the strongest negative correlations were recorded in spring with ejaculate volume (r = -0.238) and the percentage of live spermatozoa (r = -0.231), and in summer with sperm motility (r = -0.227). The association between estradiol and semen quality was generally weak across these seasons, with the strongest correlation in summer, linked to sperm motility (r = -0.329).

The present study showed significant ($p<0.01$) differences in testosterone and estradiol levels in the blood serum of bulls (Tab. 1) across seasons. The highest testosterone levels were recorded in summer, lowest in autumn and winter. Similar results were obtained by other authors [Malfatti *et al.* 2006, Chacur *et al.* 2013, Nitika *et al.* 2020], who found higher testosterone levels in the serum of bulls of Italian Mediterranean buffalo and in several domestic cattle breeds e.g. Simmental, Nellore and Karan Fries in warmer months As for estradiol, both in this study and in the studies of other authors [Brown *et al.* 1991, Javed *et al.* 2000b, Al-Delemi and Kadhim 2015], the highest concentration of this hormone was recorded in the autumn-spring season, the lowest in summer.

According to Ko [2024], chronic heat stress increases estradiol synthesis while reducing testosterone, which leads to sex hormone imbalance and spermatogenesis failure. On the other hand, Nitika *et al.* [2020] observed the highest concentration of estradiol and testosterone in crossbred Karan Fries bulls during the hot and humid season. The differences presented may result from the reproductive biology of buffaloes, which have adapted to save energy, including reproductive activity during the hot season [Al-Delemi and Kadhim 2015]. The correlation between the level of these hormones in our study depended on the season (positive in summer, autumn and winter, negative in spring). Javed *et al.* [2000b] in Buffalo bulls found a negative correlation between testosterone and estradiol in their all-year long experiment.

As with sex hormones, semen parameters in this study varied significantly across seasons. (Tab. 2). The highest volume of ejaculate was obtained from bulls in autumn and winter. These results are confirmed by other studies in Nili-Ravi buffalo [Javed *et al.* 2000a]. However, significantly different results were obtained by Bhakat *et al.* [2014] finding the highest volume of ejaculate in crossbred Karan Fries bulls in summer.

The discrepancies between the results of the studies conducted by various authors also apply to the remaining sperm parameters: sperm concentration, motility and viability. In this paper, as in the studies on zebu and taurine bulls [Chacur *et al.* 2013], Holstein Friesian bulls [Murphy *et al.* 2018]; Sahiwal bulls [Bhutta *et al.* 2020], the highest concentration of sperm was recorded in summer while it was slightly lower

in autumn and winter. In other studies [Javed *et al.* 2000a, Bhakat *et al.* 2014], in Nili-Ravi buffalo (*Bubalus bubalis*) bulls and Karan Fries bulls, higher concentrations were observed in autumn and winter.

Our research also shows that sperm from semen collected in winter and spring showed significantly greater individual mobility than in the remaining seasons (Tab. 2). Other authors in different cattle breeds: Tharparkar bulls [Rajoriya *et al.* 2013], Karan Fries [Bhakat *et al.*,2014] Mithun bulls [Perumal *et al.* 2017] made similar observations, noting a decrease in sperm motility during the summer season. In contrast, Oliveira *et al.* [2006], Chacur *et al.* [2013] and Bhutta *et al.* [2020], in Nellore, Gyr, Holstein, Zebu and Taurine bulls, found an increase in sperm motility during this period.

It should be emphasized that the testicle and scrotum are very sensitive to changes in ambient temperature, which may result in spermatogenesis disorders [Januskauskas *et al.* 1995]. According to some authors, summer season favours the occurrence of abnormal sperm forms and their greater mortality [Bhakat *et al.* 2014]. In our research, the highest percentage of live sperm in bull semen was recorded in autumn, and significantly lower in winter. In a study conducted in India [Goyal *et al.* 2024], total semen volume, sperm concentration and sperm count per ejaculation in bulls (Jersey and Holstein Friesian) were highest in summer and lowest in winter.

Semen parameters showed very diverse correlations (Tab. 3). There were negative correlations between ejaculate volume and sperm concentration, their motility and percentage of live sperm. On the other hand, there were positive correlations between concentration of sperm and individual sperm motility, and percentage of live sperm.

Table 3. Correlation values between selected bull semen parameters

Parameter	Sperm concentration ($\times 10^6$/µl)	Individual sperm motility (%)	Live sperm
Ejaculate volume(ml)	-0.287 (p=0.001)	-0.072 (p=0.137)	-0.101 (p=0.036)
Sperm concentration (x10^6/µl)		0.132(p=0.006)	0.119 (p=0.013)
Individual sperm motility (%)			0.084 (p=0.080)

Table 4. Rank correlation between hormone levels and bull semen parameters depending on the season

Hormone	Season	Correlation*			
		ejaculate volume (ml)	sperm concentration ($\times 10^6$/µl)	individual sperm motility (%)	live sperm (%)
Testosterone (ng/dl)	autumn	0.047	-0.060	0.261	-0.209
	winter	-0.055	0.069	0.147	0.010
	spring	-0.238	0.003	0.051	-0.231
	summer	-0.013	-0.204	-0.227	-0.131
Estradiol (pg/ml)	autumn	0.045	0.198	0.224	0.093
	winter	0.068	-0.286	-0.056	-0.161
	spring	-0.080	-0.140	-0.118	-0.112
	summer	-0.082	-0.022	-0.329	-0.114

*All correlations were not significant.

Diverse correlation results were also obtained when analyzing the relationship between testosterone and estradiol levels in the blood serum of bulls and semen parameters in different seasons of the year (Tab. 4). The conducted studies show that in autumn, only estradiol showed positive correlations with all semen parameters. For testosterone, positive correlations were found only with sperm volume and motility.. In the remaining seasons (especially spring and summer), the vast majority of correlations were negative. The obtained results correspond to the studies of other authors [Dixit *et al.* 1985, Souza *et al.* 2011, Rajak *et al.* 2014, Nitika *et al.* 2020], who found a very low and varied correlation of sex hormones with semen characteristics. The presented literature data indicate that both semen parameters and hormone levels are subject to seasonal fluctuations, which are particularly pronounced during periods of extreme temperatures (winter and summer).

Conclusions

Seasons have an important impact on the serum levels of testosterone and estradiol and on bovine sperm parameters. No unequivocal relation was found between the levels of sex hormones and sperm characteristics. Results indicate the need of further research on the impact of the season of sperm collection on the efficiency of insemination and development of progeny.

REFERENCES

1. AL-DELEMI D.H.J., KADHIM M., 2015 - Effect of season on the some blood hormones and enzymes of Iraqi bull buffalo. ***Journal of Dairy, Veterinary & Animal Research*** 2 (5), 195-200.
2. AL-KANAAN A., KÖNIG S., BRÜGEMANN K., 2015 - Effects of heat stress on semen characteristics of Holstein bulls estimated on a continuous phenotypic and genetic scale. ***Livestock Science*** 177, 15-24.
3. BHAKAT M., MOHANTY T.K., GUPTA A.K., ABDULLAH M., 2014 - Effect of season on semen quality of crossbred (Karan Fries) bulls. ***Advances in Animal and Veterinary Sciences*** 2(11), 632-637.
4. BHUTTA M. F., TARIQ M., TUNIO M.T., SUFYAN A., RAUF H.A., JAVED M., IMRAN S., 2020 - Season induced changes in seminal characteristics of sahiwal breeding bulls. Advances in ***Animal and Veterinary Sciences*** 8(6), 601-607.
5. BOHMANOVA J., MISZTAL I., TSURUTA S., NORMAN H.D., LAWLOR T.J., 2005 - National genetic evaluation of milk yield for heat toler-ance of United States Holsteins. ***Interbull Bulletin*** 33, 160-162.
6. BOHMANOVA J., MISZTAL I., COLE J.B., 2007 - Temperature-humidityindices as indicators of milk production losses due to heat stress. ***Journal of Dairy Science*** 90, 1947-1956.
7. BOHMANOVA J., MISZTAL I., TSURUTA S., NORMAN H.D., LAWLOR T.J., 2008 - Short Communication: genotype by environment inter-action due to heat stress. ***Journal of Dairy Science*** 91, 840-846.
8. BROWN J.L., WILDT D.E., RAATH J.R., DE VOS V., HOWARD J.G., JANSSEN D.L., CITINO S.B., BUSH M., 1991 - Impact of season on seminal characteristics and endocrine status of adult free ranging African buffalo (*Syncerus caffer*). ***Journal of Reproduction and Fertility*** 92(1), 47-57.
9. BURNS B.M., REID D.J., TAYLOR J.F., 1997 - An evaluation of growth and adaptive traits of different cattle genotypes in a subtropical environment. ***Australian Journal of Experimental Agriculture*** 37, 399-405.

10. CHACUR M.G.M., MIZUSAKI K.T., FILHO R.A.G., OBA E., RAMOS A.A., 2013 - Seasonal effects on semen and testosterone in zebu and taurine bulls. ***Acta Scientiae Veterinariae*** 41, 1110.
11. DARREN G., PAUL M., 2024 - IBM SPSS Statistics 29 Step by Step. A Simple Guide and Reference. DOI https://doi.org/10.4324/9781032622156. eBook ISBN 9781032622156.
12. DIKMEN S., HANSEN, P.J., 2009 - Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? ***Journal of Dairy Science*** 92, 109-116.
13. DIXIT N.K., AGARAL S.P., AGASWAL V. K., DWARAKNATH P.S., 1985 - Seasonal variation in serum levels of steroid hormones and their relation with seminal quality and libido in buffalo bulls. ***Theriogenology*** 24, 293-303.
14. FAROOQ U., IJAZ A., AHMAD N., REHMAN H., ZANEB H., 2013 - Seasonal variations in certain physical and biochemical attributes of semen from Cholistani bulls. ***Pakistan Veterinary Journal*** 33(4), 510-514.
15. GODFREY R.W., RANDEL R.D., FORREST D.W., SENGER P.L., 1985 - The Concentration of Estradiol-17β in Bovine Semen. ***Journal of Animal Science*** 60(3), 760-765.
16. GOSWAMI S.C., MEHTA S.N., GEORGIE G.C., DIXIT V.P., LOHAN I.S., KAKER M.L., 1991 - Effect of meteorological factors and seasons on luteinizing hormone, testosterone and semen of zebu-taurus bulls. ***Indian Journal of Animal Sciences*** 61(6), 579-583.
17. GOYAL M, TRIPATHI R, SHARMA PC, 2024 - Seasonal variations in semen traits of exotic cattle bulls of different age groups in the subtropical region. ***Indian Journal of Physiology and Allied Sciences*** 76(1), 52-56.
18. HELBIG L., WOODBURY M.R., HAIGH J. C., COLLINS J., BARTH A.D., 2007 - The seasonal fertility of North American bison (*Bison bison*) bulls. ***Animal Reproduction Science*** 97(3-4), 265-277.
19. HOFFMANN I., 2010 - Climate change and the characterization, breeding and conservation of animal genetic resources. ***Animal Genetics*** 41, 32-46.
20. JANUSKAUSKAS A., GIL J., RODRIGUEZ-MARTINEZ H., SÖDERQUIST L., LUNDEHEIM N., 1995 - Effects of a brief elevation of scrotal temperature on the post-thaw viability of bull semen. ***Reproduction in Domestic Animals*** 30, 271-277.
21. JAVED M.T., KHAN A., KAUSAR R., 2000a - Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. ***Veterinarski Arhiv*** 70(2), 83-94.
22. JAVED M. T., KHAN A., ALI M., 2000b - Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal buffalo bulls and their relationship with other semen parameters. ***Veterinarski Arhiv*** 70 (3), 141-149.
23. KO, S.-H., 2024 - Effects of Heat Stress-Induced Sex Hormone Dysregulation on Reproduction and Growth in Male Adolescents and Beneficial Foods. ***Nutrients*** 16, 3032.
24. KOWALCZYK A., CZERNIAWSKA-PIĄTKOWSKA E., KUCZAJ M., 2020 - Buserelin acetate reduces mortality and DNA defragmentation of bovine sperm cells exposed to oxidative stress. ***Pakistan Journal of Zoology*** 52(5), 1795-1799.
25. LAUDAT A., GUECHOT J., PALLUEL A.M., 1998 - Seminal androgen concentrations and residual sperm cytoplasm. ***Clinica Chimica Acta*** 276(1), 11-18.
26. MANDAL D.K., NAGPAUL P.K., GUPTA A.K., 2000 - Seasonal variation in seminal attributes and sexual behaviour of Murrah buffalo bulls. ***Indian Journal of Dairy Science*** 53(4), 278-283.
27. MALFATTI A., BARBATO O., TODINI L., TERZANO G.M., DEBENEDETTI A., BORGHESE A., 2006 - Blood testosterone levels in Italian Mediterranean buffalo bulls managed in two different breeding conditions. ***Theriogenology*** 65(6), 1137-1144.
28. MATHUR A.K., TYAGI, S., SINGH S.P., 2002 - Frieswal bull an experience of HF with Sahiwal. ***Journal of Livestock and Poultry Production*** 18(1/2), 21-23.

29. MIZERA A., KUCZAJ M., SZUL A., JĘDRASZCZYK J., 2019a - Influence of addition of cobalamin to the extender on the post-thaw motility, viability, and DNA integrity of bovine ejaculate. ***Medycyna Weterynaryjna*** 75(3), 164-168.
30. MIZERA A., KUCZAJ M., SZUL A., JĘDRASZCZYK J., 2019b - Effect of addition of buserelin acetate to the extender on motility and viability of bovine spermatozoa. ***Animal Biotechnology*** 30(2), 99-104.
31. MURPHY E.M., KELLY A.K., O'MEARA C., EIVERS B., LONERGAN P., FAIR S., 2018 - Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. ***Journal of Animal Science*** 96(6), 2408-2418.
32. NITIKA S.J., PANTH S., PANDITA S., ROY A.K., ASHUTOSH M., MOHANTY T.K., BHAKAT M., PUNETHA M., GUPTA D., 2020 - Seasonal variations in hormones and enzymes of seminal plasma and its relationship with semen quality in crossbred cattle bulls. ***Biological Rhythm Research*** 51(4), 633-643.
33. OLIVEIRA K.M., DUARTE A.M., NASCIMENTO M.R.B.M., DINIZ E.G., TAVARES M., VIEIRA R.C., 2006 - Influence of the rainy and dry seasons on the seminal characteristics of Nellore, Gyr and the Holstein bulls raised on field. ***Veterinária Notícias*** 12(2), 145-151.
34. PERUMAL P., SAVINO N., SANGMA C.T.R., KHAN M.H., EZUNG E., CHANG S., SANGTAM T.Z.T., 2017 - Seasonal effect on physiological, reproductive and fertility profiles in breeding mithun bulls. ***Asian Pacific Journal of Reproduction*** 6(6), 268-278.
35. PRAYAGA K.C., BARENDSE W., BURROW H.M., 2006 - Genetics of Tropical Adaptation. 8th World Congress on Genetics Applied to Livestock Production, August 13-18, 2006, Belo Horizonte, Brazil.
36. RAJAK S. K., KUMARESAN A., GAURAV M.K., LAYEK S.S., MOHANTY T.K., ASLAM M.K.M., TRIPATHI U.K., PRASAD S., DE S., 2014 - Testicular Cell Indices and Peripheral Blood Testosterone Concentrations in Relation to Age and Semen Quality in Crossbred (Holstein Friesian × Tharparkar) Bulls. ***Asian-Australasian Journal of Animal Sciences*** 27(11), 1554-1561.
37. RAJORIYA J.S., PRASAD J.K., GHOSH S.K., PERUMAL P., KUMAR A., KAUSHAL S., SINGH M.R., 2013 - Effects of seasons on enzymatic changes and cholesterol efflux in relation to freezability in Tharparkar bull semen. ***Asian Pacific Journal of Reproduction*** 2(4), 280-288.
38. RAVAGNOLO O., MISZTAL I., 2002 - Effect of heat stress on nonreturnrate in Holsteins: fixed-model analyses. ***Journal of Dairy Science*** 85, 3101-3106.
39. REKWOT P. I., LAMIDI O.S., ADAMU A.M., EGBUEDO C.U., RUWAAN J.S., OKEREKE S.N., 1987 - Reproductive performance of Bunaji bulls grazing natural pasture and receiving supplement containing palm karnel meal. ***Nigerian Veterinary Journal*** 18, 26-36.
40. SHATAB M.S., KUMAR A., HONPARKHE M., SINGHAL S., KAUR S., BRAR P.S., 2016 - Endocrine Status of Serum Testosterone, Estrogen and Thyroid Hormones in High Fertility Breeding Buffalo Bulls and their Male Calves. ***Journal of Animal Research*** 6(6), 1073.
41. SINGH P., RAINA V.S., 2000 - Effect of caffeine, cAMP and cattle seminal plasma on freezability of buffalo bull semen. ***Asian Australasian Journal of Animal Sciences*** 13 (7), 901-905.
42. SOUZA L.W.O., ANDRADE A.F.C., CELEGHINI E. C.C., NEGRÃO J.A., DE-ARRUDA R.P., 2011 - Correlation between sperm characteristics and testosterone in bovine seminal plasma by direct radio immunoassay. ***Revista Brasileira de Zootecnia*** 40(12), 2721-2724.
43. ZUMBACH B., MISZTAL I., TSURUTA S., SANCHEZ J.P., AZAIN M., HERRING W., HOLL J., LONG T., CULBERTSON M., 2008 - Genetic components of heat stress in finishing pigs: development of a heat load function. ***Journal of Animal Science*** 86, 2082-2088.