

**EFFECT OF SEASON ON TESTICULAR
TEMPERATURE, HORMONAL PROFILE AND SEMEN
QUALITY IN MURRAH BUFFALO BULLS**



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF**

MASTER OF VETERINARY SCIENCE

IN

LIVESTOCK PRODUCTION MANAGEMENT

BY

**TEJESHWARI SATPUTE
(B.V. Sc. & A.H)**

**DIVISION OF LIVESTOCK PRODUCTION MANAGEMENT
ICAR - NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)**

KARNAL-132001 (HARYANA), INDIA

2021

Reg. No. 19-M-LP-11

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Approved by:



**Dr. R. POUROUCHOTTAMANE
EXTERNAL EXAMINOR**



**Dr. PAWAN SINGH
MAJOR ADVISOR AND CHAIRMAN**

Members of the Advisory Committee

1. Dr. T.K. Mohanty

Principal Scientist, ARGO

2. Dr. Sohanvir singh

Principal Scientist, Animal Physiology

3. Dr. Mukesh Bhakat

Principal Scientist, LPM

4. Dr. Rakesh Kumar

Principal Scientist, ABTC
(Jt. Director's Nominee)



18/9/2021





LIVESTOCK PRODUCTION AND MANAGEMENT DIVISION
ICAR- NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL-132001 (HARYANA), INDIA



Dr. PAWAN SINGH

(Principal Scientist and In-charge ABRC)

ICAR-NDRI, Karnal-132001, Haryana, India

CERTIFICATE

This is to certify that the thesis entitled, “**EFFECT OF SEASON ON TESTICULAR TEMPERATURE, HORMONAL PROFILE AND SEMEN QUALITY IN MURRAH BUFFALO BULLS**” submitted by **TEJESHWARI SATPUTE** in the partial fulfillment of the award of the degree of **MASTER OF VETERINARY SCIENCE IN LIVESTOCK PRODUCTION MANAGEMENT** of the ICAR-National Dairy Research Institute (Deemed University), Karnal (Haryana), is a bonafide research work carried out by her under my supervision and no part of the thesis has been submitted for any other degree or diploma.

Dated: 09/09/2021

Dr. PAWAN SINGH

MAJOR ADVISOR & CHAIRMAN



**Dedicated
To My
Beloved Parents
&
Respected Guide**

ACKNOWLEDGEMENT

Every victory is hard earned, and it is not a one man job. It is always accomplished by aid and encouragement by a lot of teammates and mentors. This work is also a consequence of hard work, care, guidance and critical appraisal of so many people, who were alongside me in every step of my life. Words are insufficient to express the emotions and therefore my acknowledgements are many times more than what I am expressing.

“Beginning with the god the Almighty”

*With proud gratitude and heartfelt indebtedness, first and foremost, I would like to express my deep sense of obligation and gratefulness to **Dr. Pawan Singh**, Principal Scientist and In-charge, ABRC, Karnal. My major advisor and chairperson of advisory committee Sir, you have been a tremendous mentor for me. Without your encouragement and guidance this project would not have materialized. Your meticulous guidance, constant supervision, affectionate encouragement, personal interest, your patience, time devoted for my growth as a research scholar are priceless. It was a great pleasure and a matter of privilege to work under your guidance during my master’s degree programme. I thank you for each and everything you taught me during these days.*

*I am extremely thankful to **Dr. Sohanvir singh**, Principal Scientist, Animal Physiology and member of the advisory committee who provided a persistent support, keen interest, constructive criticism, persuasion and unstinting support, all the advice and help rendered to me during my research work.*

*I owe my sincere thanks to **Dr. T.K. Mohanty**, Principal Scientist, ARGO and member of the Advisory Committee, for his valuable guidance, shrewd suggestions, expert advice and gentle support during my entire research work. He has remained extremely approachable and friendly throughout the course of my study.*

*I am cordially indebted to members of the advisory committee **Dr. Mukesh Bhakat** Principal Scientist, Livestock Production Management Division and **Dr. Rakesh Kumar** Principal Scientist, ABTC for their never ending support and sustained interest, propitious help for framing up and successful completion of this research work.*

*I feel the honor to express my deep reverence to **Dr. R.R.B. Singh**, former Director and **Dr. M.S. Chauhan**, Director, ICAR-NDRI, Karnal, for providing the necessary research and academic facilities required for my study and throughout the tenure of my research work. I express my gratitude to ICAR for keeping my financial worries at bay in the form of Institutional fellowship.*

*I acknowledge with profound gratitude the support and help from **Dr. Manishi Mukesh** and **Dr. R. S. Kataria**, Principal Scientists, NBAGR, Karnal and **Dr. Nishant kumar**, Scientist, ARGO, NDRI for the successful conduct of this project.*

*I take great pleasure in thanking **Dr. A. K. Mishra**, Principal Scientist (Incharge & Head LPM Division), **Dr. M. L. Kamboj**, Principal Scientist, Livestock Production Management Division, **Dr. S. S. Lathwal**, Principal Scientist, Livestock Production Management Division and **Dr. Ramesh Chandra**, Senior Scientist, Livestock Production Management Division, **Dr. Rubina Kumari Baithalu**, Scientist, ARGO, NDRI for their support throughout the research work.*

*The help and co-operation of my dearest seniors Dr's. **Sriranga K. R.**, **Jaya Karosiya**, **Ritu Badgujjar**, **Anusmita Baishya**, **Gayathri G. N.** and **Manmohan singh Rajpoot** was instrumental in the completion of my research work.*

*I express my heart full of thanks to my dear friends Dr's. **Parinita Prashar**, **M. Sravani Reddy**, **Divyanshu Singh Tomar**, **Sheetal More**, **Sushmita Baishya** and **Dipti Nain** for their friendly support and endless help offered all along my stay at NDRI, Karnal.*

*My long stay at NDRI was made memorable by the good company of all mybatch mates. Thank you Dr's. **Atul Singh Rajpoot**, **Smaranika**, **Haroon**, **Asheq, kiran**, **Ajay**, **Sangram**, **Chomba**, **Sukankshi** and **Parul** for their kind help and cooperation during my whole two years.*

*Warm thanks are extended to my seniors Dr's **Gautam Kumar**, **Suresh**, **Sadhna Tiwari Nishtha Kushwaha** and **Vishakha Uttam** for their support and help rendered.*

*I express my sincere thanks to all the faculty and staff members of Artificial Breeding Research Centre. Especially I would like to thank **Dr. Kaushal Kumar**, **G.S. Meena**,*

Rajesh Khara, Shyam singh ji. Ganesh ji. Sandeep Tanwar, Bintu Burman, Gaurav for extending a helping hand, cooperation and making my stay memorable at ABRC.

I wish to express my deep reverence to Dr. Prince Vivek, RA, ABRC, Karnal for providing necessary support.

I have no words to express my gratitude and sincere thanks to **Kamlesh mam, Shiv Kumar ji, Gurpreet ji and Nafe ji** whose cooperation was always with me during my research work. I sincerely thank **Sohan bhaiya, Rakesh bhaiya, Rajesh bhaiya, Prem bhaiya, Suraj bhaiya, Raju bhaiya, Sonu bhaiya** and entire workforce of ABRC whose support, cooperation and hard labour for care and management of animals made my study possible on time.

Words are inadequate to express my indebtedness and deep sense of honor to my affectionate parents **Mr. C. R. Satpute and Mrs. Poornima Satpute**, my sister **Naina di**, and Brothers **Gopal and Devendra** and my dearest mama **Dr. R. K. Naitam** for their overwhelming love, constant encouragement, powerful support and efforts which helped me to successfully complete the course.

Last, but not least I am grateful to all Murrah buffalo Bulls, without them this research would not have come into existence.

Date – 09/09/2021


Tejeshwari Satpute
(satputetejeshwari@gmail.com)

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ABBREVIATIONS

%	Percentage
°F	Degree Fahrenheit
°C	Degree Celsius
ad lib	Ad libitum
AI	Artificial Insemination
AM	Ante Meredian
ANOVA	Analysis of variance
Db	Dry Bulb
DPT	Distal Pole Temperature
<i>et al.</i>	Co-workers
Fig.	Figure
g	Gram
GH	Growth Hormone
HOST	Hypo Osmotic Swelling Test
i.e.	That is
IM	Individual Motility (%)
IRT	Infrared Thermography
L	Liter
MA	Mass Activity
mg	Milligram
mg/dl	Milligram per decilitre
mg/ml	Miligram per mililiter
Min	Minute
min.	Minute
ml	Mililiter

mmol	Milimole
MPT	Middle Pole Temperature
NDRI	National Dairy Research Institute
ng	Nano gram
nm	Nanometer
No.	Number
PM	Post Meridian
PR	Pulse Rate
ROS	Reactive Oxygen Species
RR	Respiration Rate
RT	Rectal Temperature
SE	Standard Error
TG	Testicular Gradient
THI	Temperature Humidity Index
Viz.	Namely
Wb	Wet bulb
Wt	Weight
μg	Microgram
μl	Micro Litre

ABSTRACT

Artificial insemination has been proved as the most rapid and cost effective method to improve the productivity of livestock including buffaloes, as a result the demand for frozen semen of elite bull is increasing day by day in the country. The quality semen from high genetic merit bulls is imperative in improving the productivity of buffaloes. In tropical and sub-tropical regions heat stress is the major constraint for buffalo bull's performance, which is contributed by number of factors like temperature, humidity, rainfall, photo-period etc. However, how heat stress affects buffalo bulls performance is not understood yet, therefore, the present study was planned to investigate the effect of season on testicular temperature, hormonal profile and semen quality in Murrah buffalo bulls. The experimental period was divided into three seasons- winter (15th December to 15th February), spring (16th February to 30th April) and summer (1st May to 30th June). For that 14 mature Murrah buffalo bulls of ~3-4 yrs age were selected. Physiological responses and surface temperature (Infrared thermal imaging) at eye, forehead, flank, rump and scrotal surface were recorded during morning and afternoon hrs. Semen was collected twice a week and sexual behaviour was recorded at the time of collection. Semen quality parameters were evaluated two times during peak of each season. Blood for hormone estimation was collected on the day of semen collection. Data so generated was analysed by one-way analysis of variance (ANOVA) using SPSS software. The results showed that the ambient temperature and THI was higher during summer season as compared to winter and spring. The rectal temperature (°C) and respiration rate in morning and afternoon hours was least during winter and higher ($p < 0.05$) in summer season. Body surface temperature (eye, Forehead, flank and Rump region) showed difference ($p < 0.05$) among seasons, being lowest in winter and highest in summer. The testicular temperature gradient (°C) was higher ($p < 0.05$) in the morning during spring season than summer season. Activity of thyroid gland in terms of T3, T4 and TSH concentration was lower ($p < 0.05$) in summer as compared to winter and spring season. Conversely, testosterone concentration was higher in summer season; however, cortisol concentration did not vary significantly among seasons. The bulls have shown short reaction time and higher libido score during spring season as compared to other seasons. Higher ($p < 0.05$) values of sperm concentration, mass motility, individual motility and HOST reacted spermatozoa was obtained in spring and lowest during summer except for acrosome integrity. So from this study, it can be concluded that the season has significantly influenced the physiological and endocrinological responses, sexual behavior and overall semen quality of Murrah buffalo bulls.

मुर्रा सांडों में वृषण के तापमान, हार्मोनल प्रोफाइल और वीर्य की गुणवत्ता पर मौसम का प्रभाव

सारांश

पशुधन की उत्पादकता में सुधार के लिए कृत्रिम गर्भाधान सबसे तेज और प्रभावी तकनीक साबित हुई है, जिसके परिणामस्वरूप पूरे देश में उच्च गुणवत्ता वाले सांड के हिमीकृत वीर्य की मांग दिन प्रतिदिन बढ़ती जा रही है। भैंसों की उत्पादकता में सुधार के लिए उच्च आनुवंशिक योग्यता वाले सांडों का गुणवत्तापूर्ण वीर्य अनिवार्य है। उष्णकटिबंधीय और उपोष्णकटिबंधीय क्षेत्रों में गर्मी भैंस के सांड की प्रजनन क्षमता के लिए प्रमुख तनाव है। तापमान, आर्द्रता और वर्षा, आदि जैसे विभिन्न कारकों से सांड की प्रजनन क्षमता में बाधा आती है। इसलिए वर्तमान अध्ययन की योजना मुर्रा सांडों में वृषण के तापमान, हार्मोनल प्रोफाइल और वीर्य की गुणवत्ता पर मौसम के प्रभाव की जांच करने के लिए की गई है। जलवायु परिस्थितियों के आधार पर प्रयोग अवधि को तीन मौसमों - सर्दी (15 दिसंबर से 15 फरवरी), वसंत (16 फरवरी से 30 अप्रैल) और गर्मी (1 मई से 30 जून) में विभाजित किया गया। इसके लिए करीब 3-4 साल के मुर्रा सांडों का चयन किया गया। शारीरिक प्रतिक्रियाओं और शरीर के विभिन्न भागों जैसे आंख, माथे, कोख, पुट्टे और अंडकोश के सतह तापमान (इन्फ्रारेड थर्मल इमेजिंग) को सुबह और दोपहर के दौरान दर्ज किया गया। तापमान प्रवणता (TG) की गणना ऊपरी और निचले अंडकोश सतह के तापमान के बीच के अंतर से की गई। सप्ताह में दो बार वीर्य एकत्र किया गया और वीर्य संग्रह के समय यौन व्यवहार लिया गया। वीर्य संग्रह के दिन हार्मोनल विश्लेषण के लिए रक्त एकत्र किया गया। सर्दी, वसंत और गर्मी के मौसम के दौरान दर्ज परिवेश का न्यूनतम तापमान (°C) सुबह का 9.43 ± 0.43 , 17.99 ± 0.52 और 26.6 ± 0.29 और दोपहर का 19.56 ± 0.63 , 32.19 ± 0.58 और 34.87 ± 0.52 पाया गया। सर्दियों में न्यूनतम और गर्मियों में सबसे अधिक (पी < 0.05) तापमान दर्ज किया गया। टीएचआई गर्मियों में (80.79 ± 0.38), वसंत (72.36 ± 0.5) और सर्दी (58.01 ± 0.75) दर्ज किया गया। सर्दी, वसंत और गर्मी के दौरान सुबह मलाशय का तापमान (°C) क्रमशः 36.91 ± 0.05 , 37.27 ± 0.05 और 37.58 ± 0.05 पाया गया। यह अन्य मौसमों की तुलना में गर्मियों में अधिक (p < 0.05) था। इसी प्रकार श्वसन दर सर्दियों में कम (11.88 ± 0.12) एवं गर्मी में के मौसम में अधिक (24.38 ± 0.42) पाया गया। शरीर के विभिन्न अंगों के तापमान (आंख, माथे, कोख और पुट्टे) के लिए दर्ज किए गए डेटा में अंतर (पी < 0.05) देखा गया, जिसमें सबसे कम सर्दियों में तथा गर्मियों में उच्चतम दर्ज किया गया। सर्दियों, वसंत और गर्मियों के दौरान सुबह का तापमान प्रवणता (डिग्री सेल्सियस) क्रमशः 5.61 ± 0.16 , 6.63 ± 0.25 और 37 ± 0.23 पाया गया, वसंत ऋतु के दौरान उच्चतम (पी < 0.05) तापमान प्रवणता दर्ज की गयी। सर्दियों, वसंत और गर्मी के मौसम में T3 (ng/mL) सांद्रता 4.98 ± 0.21 , 4.28 ± 0.14 और 1.79 ± 0.31 थी, जो सर्दियों के मौसम में अधिक और गर्मी के मौसम में कम थी। अध्ययन के विभिन्न मौसमों के दौरान औसत TSH (μ IU/mL) सांद्रता गर्मियों की तुलना में सर्दियों और वसंत ऋतु में काफी अधिक थी। वर्तमान अध्ययन में सर्दियों के महीनों के दौरान कोर्टिसोल (ng/mL) सांद्रता लगभग बराबर थी और वसंत (1.46 ± 0.18) की तुलना में गर्मियों (2.41 ± 0.37) के दौरान टेस्टोस्टेरोन (ng/mL) सांद्रता के लिए औसत मान काफी अधिक था। गर्मी के दौरान प्रतिक्रिया समय और स्खलन के लिए लिया गया कुल समय गर्मियों के दौरान लंबा और वसंत के मौसम के दौरान कम था। मुर्रा सांडों का कामेच्छा स्कोर (LS) वसंत (38 ± 1.72) के दौरान गर्मियों (29.17 ± 3.04) की तुलना में अधिक था। वर्तमान अध्ययन में शुक्राणु संख्या, द्रव्यमान, गतिशीलता, व्यक्तिगत गतिशीलता और HOST प्रतिक्रिया वाले शुक्राणु के लिए उच्च (पी < 0.05) मान वसंत में और गर्मियों के दौरान सबसे कम देखा गया। इस अध्ययन से यह निष्कर्ष निकाला जा सकता है कि मौसम शारीरिक प्रतिक्रियाओं, यौन व्यवहार और सांडों में वीर्य की गुणवत्ता में एक प्रमुख भूमिका निभाता है।

CHAPTER -1

Introduction

INTRODUCTION

Buffalo is an important component of Indian livestock, as it is the main source of milk, meat, and drought power. Present buffalo population in the country is 109.85 million; which constitutes 20.5% of the total livestock population. India is the largest milk producer country in the world with the production of 198.40 million tonnes (DAHD Annual Report, 2020-21), out of which 49% is contributed by buffalo (DAHD Annual Report, 2019-20).

Artificial insemination technique is being used widely to increase the genetic potential for the production of livestock species including buffalo all over the world. Quality semen from high genetic merit bulls is imperative in improving the fertility and productivity of buffaloes. The environmental variables associated with heat stress which affects the physiological responses and thermal regulation is primarily ambient temperature and relative humidity. In tropical and sub-tropical areas, heat stress is the major constraint on animal productivity (Marai *et al.*, 2010). The temperature-heat index (THI), a most commonly used measure to detect the heat stress in cattle (Gaughan *et al.*, 2008). It is reported that the cow suffer from heat stress when THI exceeds above 72 (Ingraham *et al.*, 1974). Sinha (2018) also reported that the Murrah buffalo bulls faced most stressful condition during rainy and summer season when THI was maximum. Season plays an important role in the reproduction and quality of semen (Koonjaenak *et al.*, 2007). In bulls, heat stress causes several undesirable changes in characteristics of semen quality and quantity by affecting spermatogenesis and epididymal maturation of spermatozoa (Meyerhoeffer *et al.*, 1985), as well as ejaculation and mating behavior (Titto *et al.*, 2011). The effect of season on bovine semen production has been studied extensively (Stalhammar *et al.*, 1989; Brito *et al.*, 2002; Snoj *et al.*, 2013; Bhakat *et al.*, 2014; Malama *et al.*, 2017) yet the information on the effect of season on bull semen characteristics has been reported to be variable. Some researchers have found a significant effect of the season (Bhakat *et al.*, 2015; Alam *et al.* 2015; de Castro *et al.*, 2017), while others have found a non-significant effect (Mandal *et al.*, 2000; Helbig *et al.*, 2007). Among various climatic factors, heat stress combined with higher relative humidity is the major source of sperm quality reduction during spermatogenesis (Marai *et al.*, 2007; Dikmen and Hansen, 2009). Hot-dry or summer season has been reported to

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be unfavourable for reproduction in Murrah buffalo bulls (Bhakat *et al.*, 2015; Sinha, 2018). The percentage of total morphologically abnormal spermatozoa and the percentage of spermatozoa with bent tail and distal cytoplasmic droplet increased in high THI as compared to lower THI (Seifi-jamadi *et al.*, 2020).

High ambient temperature compromises the thermoregulatory mechanism of body and heat dissipation, which causes thermal stress in animals (Marai *et al.*, 2009). Respiration rate, rectal temperature, pulse rate, and skin temperature are the major physiological parameters that alter in animals during thermal stress (Lakhani *et al.*, 2018). Testicular temperature plays an important role in male reproduction (Rizzoto and Kastelic, 2020). Maintenance of temperature of testicles around 32°C is important for proper spermatogenesis (Morrell, 2020) and it depends on the testicular thermoregulation of bulls which is highly sensitive to slight changes in scrotal temperature (Boni, 2019). The testicular temperature in bulls must be 2 to 6 °C below core body temperature for normal spermatogenesis (Skinner and Louw 1966; Mieusset and Bujan, 1995). Testicular thermoregulation is maintained by localization of the testes outside the abdominal cavity in the scrotum. The skin of the scrotum is thin, fairly hairless, and devoid of subcutaneous fat which aids in thermoregulation. The subcutaneous tissue of the scrotum has extensive blood and lymphatic supply which facilitates radiation of heat (Blazquez *et al.* 1988). Cremaster muscle and tunica dartos contract in cold environment to draw the testes closer to the body and relaxes in hot environment to keep the testis away from the body (Setchell, 1978). Increased testicular temperature leads to an increase in oxygen demand owing to increased metabolism in the testes which is responsible for the increase in testicular blood flow and O₂ delivery (Rizzoto *et al.*, 2020). When testicular blood flow is not able to meet the increased demand for oxygen, testes become more hypoxic in response to the heat stress and lead to increased production of reactive oxygen species (ROS) as byproducts of metabolism and consequently, causes oxidative stress (Setchell, 1998). Increased ROS concentration in cells and tissues in response to heat stress negatively affects reproductive functions in mammals (Boni, 2019). Sperm cells are highly susceptible to damage caused by ROS due to the presence of large amounts of polyunsaturated fatty acids in the plasma membrane and low antioxidant enzymes concentrations in the cytoplasm (Nichi *et al.*, 2006). ROS damages lipid, protein, and DNA by affecting the sperm mitochondrial electron transport chain (ETC) which results in apoptosis or normal cell death (Sanocka and Kurpisz, 2004; Diebold and Chandel,

2016). Lipid peroxidation resulting from ROS affects sperm motility, viability (Rahman *et al.*, 2011), sperm membrane fluidity, and also affects sperm cryo-survival (Aitken and Curry, 2011). ROS generated due to heat stress may act as a cell death signal responsible for increased apoptosis of spermatozoa (Peris *et al.*, 2007).

Studies regarding seasonal variation in semen characteristics have mainly attributed these changes to poor heat dissipation mechanisms and compromised scrotal thermoregulation (Menegassi *et al.*, 2015) as well as the alteration in endocrine profile of the animal and the differential response of bull testes to gonadotropins (Jimenez-Severiano *et al.*, 2003). Among all environmental variables, photoperiod is the main regulator of reproduction in buffalo (Michael *et al.*, 2020). Buffalo is a negatively photoperiodic animal that shows a natural increase in fertility in response to decreasing day length (Wankhede *et al.*, 2015) with the lowest breeding activity during the summer season and highest in the autumn and winter season (Heuer *et al.*, 1987). Major hormones which are responsible for thermal adaptation are thyroid hormone, cortisol, growth hormone, prolactin, insulin, and aldosterone (Mishra, 2021). Seasonal variations associated with testosterone concentrations and level of luteinizing hormone and melatonin hormones can affect spermatogenesis (Godfrey *et al.*, 1990; Lincoln *et al.*, 1996; Tatman *et al.*, 2004). Thermal stress with an increase in THI directly stimulates the hypothalamo-pituitary-thyroid (HPT) axis and causes a decrease in the secretion of thyrotropin stimulating hormone (TSH) and in turn triiodothyronine (T3) and thyroxine (T4) hormone secretion (Magdub *et al.*, 1982; El Nouty and Hassan, 1983; Fregly, 1989; Chaudhary *et al.*, 2015). Thyroid hormone plays a major role in metabolism, growth (Mayahi *et al.*, 2014), seminal quality, and reproduction in domestic animals (Warwick *et al.*, 1948; Maqsood, 1952; Goswami, 1962). Activation of the hypothalamo-pituitary-adrenal (HPA) axis due to heat stress leads to the secretion of corticotrophin releasing hormone (CRH) from the hypothalamus which acts on corticotrophs of the anterior pituitary to secrete adreno-corticotrophic hormone (ACTH) which finally stimulates the adrenal cortex to secrete cortisol into the circulation (Minton, 1994). The pituitary hypothalamus-gonadal axis regulates the secretion of GnRH, which in turn regulates the secretion of gonadotrophins and testosterone hormone required for spermatogenesis, sperm maturation, and reproductive behavior in bulls (Kowalczyk *et al.*, 2021). Heat stress affects the hypothalamic-pituitary-gonadal axis and inhibits the secretion of gonadotropin-releasing hormone (GnRH). In the anterior hypophysis it interferes with

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the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) and in the gonads alters the secretion of sex steroids (River and Rivest, 1991). Irregular secretion of these reproduction hormones exerts adverse effects on testosterone production, libido score, and sperm production in males. The major androgen necessary for spermatogenesis is testosterone; it is responsible for the maintenance of secondary sexual characteristics, libido and promotes sperm production in bulls (Hafez and Hafez, 2013).

Though few scattered and independent attempts have been made to assess the effect of different seasons of a year on testicular temperature, hormonal profile, semen quality and their interrelationship in buffalo bulls; however, a complete and systematic information is very less. In view of the need to address the aforementioned issues in a holistic manner, the current study was planned to investigate the effect of season on testicular temperature, hormonal profile, and semen quality in Murrah buffalo bulls with the following objectives:

1. To study the effect of season on physiological responses and hormonal profile in Murrah buffalo bulls.
2. To investigate the effect of different season on sexual behaviour and semen quality in Murrah buffalo bulls.

CHAPTER -2

Review of Literature

REVIEW OF LITERATURE

Seasonal variations in environmental parameters such as temperature, humidity, and radiations have a negative impact on livestock's development, productivity, and reproduction. Low and high ambient temperatures create discomfort and increase stress, which leads to a decrease in the animal's physiological and metabolic processes. The function of bulls' testes, epididymis and other parts of the genital tract, including accessory sex glands is reflected in the quality of their semen. The bull's genital system's normality is also dependent on environmental variation and hormonal balance, since it is vulnerable to changes in health, diet, and management. Seasonal changes in testicular temperature, hormonal profile, sexual behavior and semen quality in bulls are all represented in the ejaculate as volume, sperm concentration, and other sperm properties such as motility, morphology and viability, etc. In this chapter, literature available on the effect of season on testicular temperature, hormonal profile and semen quality in buffalo bulls has been reviewed under following sub- headings:

2.1 Effect of season on physiological parameters

The buffalo is primarily a shade and water-loving mammal that prefers to live in areas with several water streams. Air temperatures of 13–18 °C along with an average relative humidity of 55–65 %, wind speed of 5–8 km/h, and a medium intensity of sunshine are optimum climatic conditions for buffalo's growth and reproduction (Payne, 1987). When exposed to direct solar radiation or hot weather, they show signs of severe distress due to the negative effect of elevated ambient temperature on appetite and feed intake, resulting in slower development and impaired reproduction (Marai *et al.*, 2002). Buffaloes can adapt to hot ambient circumstances well, but their black colour of body (Shafie, 1985) and lack of sweat glands (Nagarcenkar and Sethi, 1981) and low hair density (Shafie and El-Khair, 1970) make them more vulnerable to thermal radiation, resulting in heat stress and hormone imbalances than zebu cattle (Silva *et al.*, 2014). Dairy animals are more susceptible to heat stress due to their higher metabolic rate and heat generated during rumen fermentation (Purwanto *et al.*, 1990). An increase in body temperature of approximately 1.0°C has been shown to have noticeable, negative consequences on metabolism, tissue integrity, and production (McDowell *et al.*, 1976). The most evident markers of heat stress in buffalo are physiological indicators. Deviation

Review of Literature

from the physiologically normal rectal temperature has been used as a measure of discomfort. In animals, rectal temperature is the most accurate and useful indicator (Brandon, 2014). Ambient temperature, solar radiation, relative humidity and wind speed also influence respiratory rate. The most important element among these has been found to be ambient temperature. As compared to the winter season, the rate of respiration per minute increases during the summer (Singh *et al.*, 2014). Evaporative cooling is used to increase heat loss through increased respiration. The higher rate of respiration in the summer compared to the winter could be attributed to the tissues' increased demand for oxygen in stressful situations. Rectal temperature is thought to be a reliable indicator of body temperature (Srikandakumar and Johnson, 2004). RT is a thermal balancing indicator that may be used to quantify the harshness of the environment (Silanikove, 2000). According to Dandage (2009) there is a positive association between RT and humidity and other physiological responses in Karan Fries cattle and Murrah buffaloes.

The effect of seasonal variation on physiological parameters in Murrah buffaloes was examined by Dayal *et al.* (2017). For the experiment, 25 healthy non-lactating Murrah buffaloes of age more than two years were chosen. The effect of heat stress on buffalo was studied by measuring rectal temperature and respiration rate in different seasons. Rectal temperature was measured with the help of mercury thermometers for each animal daily both in the morning and evening and respiration rate was measured by observing the movement of the thoracoabdominal region for each animal daily. Season had no influence on rectal temperature (winter; $100.3 \pm 0.47^{\circ}\text{F}$ and Summer; $100.2 \pm 0.23^{\circ}\text{F}$) but it did have a significant effect ($P < 0.05$) on respiration rate (winter -13.2 ± 0.4 ; Summer -16.9 ± 0.9).

Sayah *et al.* (2019) conducted research at the Sakha Experimental Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A total of 16 healthy 15-month-old Friesian young bulls were divided into two groups. Bulls were kept in two different housing systems (tie-stall and loose barn) under the same environmental conditions to assess semen quality and some physiological characteristics during three distinct seasons (winter, spring and summer). At 8:00 a.m. and 2:00 p.m., body temperature (BT) was taken twice a day. The results revealed that BT was strongly influenced ($P < 0.05$) by season, with the highest in summer (35.26°C), moderate in spring (33.93°C) and the lower in winter (33.44°C).

Singh *et al.* (2014) investigated the differences in physiological parameters in Murrah buffaloes during different seasons. Six 18-24 month old Murrah buffalo heifers were chosen for the experiment. The heart rate (HR), respiration rate (RR), and rectal temperature all were monitored. During the summer and winter, the rectal temperature was $102.52 \pm 0.25^{\circ}\text{F}$ and $100.68 \pm 0.19^{\circ}\text{F}$, respectively and respiration rates were 77 ± 1.25 and 12.29 ± 1.97 breaths per minute, respectively. During the summer and winter seasons, there was a significant difference in respiration rate ($P < 0.001$) and rectal temperature ($P < 0.05$) and an increased respiration was considered to be an attempt to increase heat loss through evaporative cooling.

Bhan *et al.* (2013) reported that there is impact of temperature variability on physiological parameters of growing and adult Karan Fries cattle during hot humid, winter, spring and summer seasons. Respiration rate (RR) and rectal temperature (RT) was measured during forenoon and afternoon and it was observed that RT ($^{\circ}\text{C}$) was 38.25 ± 0.10 , 37.79 ± 0.10 , 38.35 ± 0.07 and 39.04 ± 0.15 during morning and 38.67 ± 0.08 , 38.67 ± 0.05 , 38.88 ± 0.05 and 39.55 ± 0.12 during afternoon in spring, winter, Hot humid and summer seasons respectively. Respiration rate (RR/min) was 20.00 ± 0.58 , 15.16 ± 0.40 , 20.50 ± 0.22 and 22.83 ± 0.79 during morning and 22.00 ± 0.63 , 18.00 ± 0.45 , 25.83 ± 0.31 and 28.67 ± 0.21 during afternoon in spring, winter, hot-humid and summer seasons respectively. The values of Respiration rate (RR) increased from 2.83 to 6.67 breaths/min and rectal temperature (RT) 0.79°C to 0.88°C respectively in adult KF cows during forenoon and afternoon in summer over spring season.

Kumar *et al.* (2017) investigated the seasonal factors affecting physiological parameters of lactating Harijana ($n=6$) and Sahiwal ($n=6$) cows raised under sub-tropical Indian conditions for summer and winter seasons. The rectal temperature and respiration rate were 102.19 ± 0.03 , 102.28 ± 0.04 and 28.71 ± 0.68 , 27.50 ± 1.00 during summer season in Harijana and Sahiwal cows respectively. Rectal temperature and respiration rate were 100.17 ± 0.03 , 100.27 ± 0.04 , and 18.00 ± 0.73 , 20.52 ± 1.05 during winter season in Harijana and Sahiwal cows respectively. Physiological responses exhibited significant ($p < 0.05$) increase during summer season as compared to winter season.

The seasonal variability in physiological, oxidative, and metabolic responses of Nili-Ravi buffaloes to hot and humid climate was investigated by Mengwei *et al.* (2021) at The Guangxi Buffalo Research Institute in Nanning, China. For this investigation, 20 non-lactating multiparous buffaloes were used. Environmental variables were recorded

twice daily in the morning (at 8:00 a.m.) and afternoon (at 2:30 p.m.) and used to show monthly meteorological fluctuations over the course of the study. During the Spring, Summer, Autumn, and Winter seasons, the respiratory rate (times/min) was 13.27, 17.32, 15.16, 10.61, and rectal temperature (°C) was 38.11, 38.26, 38.18, 38.04, respectively. Body Surface Temperature (BST) and RR of non-lactating buffaloes increased significantly in the summer when the THI value reached 82, according to the findings of the study. Increased RR and pulse rate (PR) represented animals' attempts to decrease their body temperatures through evaporative cooling as an adaptive approach.

2.2 Regulation of scrotal and testicular temperature

Maintenance of testicular temperature around 32°C is important for normal spermatogenesis. High environmental temperatures, either alone or in combination with high humidity interfere with evaporative heat loss from the scrotal surface. This elevated environmental temperature leads an elevated body temperature and the high metabolic activity of the tissue which results in an elevated temperature within the testis (Morrell, 2020).

The testes and epididymis of bovines are situated in the scrotum below abdomen to maintain them at 2°C to 6°C lower temperature than core body temperature for proper functioning (Waites, 1970; Brito *et al.*, 2004). Scrotal temperature is regulated in the same way as the rest of the body there are several mechanisms which contribute to testicular cooling. In bulls, the scrotal skin is thin, relatively hairless and devoid of subcutaneous fat. It has more sweat glands than the other parts of the body which helps with heat transfer by allowing heat to be dissipated through sweating (Blazquez *et al.*, 1998). Apart from evaporation, temperature regulation in the testes occurs via blood flow. The testicular vascular cone (TVC) which is located above the testis consists of a highly coiled testicular artery surrounded by pampiniform plexus (a complex network of small veins) (Hees *et al.*, 1984). The TVC acts as a countercurrent heat exchanger to transfer heat from the testicular artery to the testicular vein for cooling of blood before it enters the testis (Brito *et al.*, 2004; Kastelic *et al.*, 2014). Smooth muscles of cutaneous arterioles of the scrotum dilate in warm condition and constrict during cold condition and helps in temperature regulation (Waites, 1970). Muscular contraction and relaxation of cremaster muscle and dartos muscle allows the testes to be drawn towards the abdomen in cold conditions and to hang away from the body in warm conditions (Morrell, 2020).

Heat stress can disturb testicular thermoregulation which leads to testicular degeneration that causes decline in sperm quality due to low sperm concentration and motility as well as high sperm abnormalities (Barth and Bowman, 1994; Kastelic *et al.*, 1996; Brito *et al.*, 2003; Rahman *et al.*, 2011; Das *et al.*, 2017).

2.3 Infrared thermography

Infrared thermography is a non-invasive remote sensing method used for measuring changes in heat transfer and blood flow, through the detection of small changes in body temperature (Kastelic *et al.*, 1997; Nääs *et al.*, 2020). Thus, this technology can be used as a general indicator of stress. Infrared thermography can be used to measure scrotal surface temperatures and thereby helps in making interpretations regarding testicular temperature. Infrared thermography cameras produce a colour image, with different colours corresponding to different temperature ranges. As a result, the scrotal surface temperature patterns can be quickly interpreted (Kastelic *et al.*, 2014). A Temperature drop from the dorsal to ventral portion of a bull's scrotum in apparently normal testicular thermoregulation can be interpreted through infrared thermography (Kastelic, 2014; Silva *et al.*, 2014).

2.4 Effect of increased testicular temperature on semen quality

Heat stress can cause serious reproductive failure in bulls (Hansen, 2009) by interfering with testicular thermoregulation. Heat stress which leads to damage of the testicular tissue (Barth and Bowman, 1994; Sabes-Alsina *et al.*, 2019) affects spermatogenesis which resulting in subfertility or infertility in bulls (Kastelic *et al.* 1997; Paul *et al.*, 2009). When the ambient temperature rises, the scrotal subcutaneous temperature gradient decreases (Kastelic *et al.*, 1996). Increased metabolism and oxygen demand results from increased testicular temperature, but due to limited blood flow this increased demand cannot be fulfilled leading to hypoxia, reactive oxygen species production, and decrease in semen quality (Setchell, 1978). Heat-induced spermatogenic cell damage is caused by oxidative stress, which results in faulty chromatin protamination and eventually spermatozoa death and DNA fragmentation (Paul *et al.*, 2008). Germ cells are highly susceptible to heat stress due to the higher cell division activity. In bovine bulls, scrotal hyperthermia damages germ cells in the meiotic phase due to an increase in reactive oxygen species and a negative impact on mitochondrial formation and function, lowering sperm motility and viability (Rahman *et al.*, 2011).

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Yadav *et al.* (2017) in their study assessed the relationship of scrotal surface temperature gradient (SSTG) with semen quality in Murrah buffalo breeding bulls. Buffalo breeding bulls (n=130) were chosen from four separate semen centers for this study. Ejaculate volume (EV), mass motility (MM), concentration (SPC), motility (SM), viability, and abnormalities (SA) were all measured in each bull's ejaculates. Individual bulls' SSTG was measured using digital Infrared thermography. The bulls with a larger temperature gradient produced higher-quality sperm than those with a smaller temperature gradient. According to the findings the mass motility, concentration varied among the groups along with SSTG.

Kushwaha *et al.* (2018) studied the effect of scrotal surface temperature gradient on semen quality attributes of Karan Fries breeding bull. During the peak winter season, six ejaculates were collected from each randomly selected bull (n=15), and seminal quality parameters were assessed while the scrotal surface temperature gradient (SSTG) was recorded using the DarviDTL007 Infrared thermography camera. Based on SSTG, three groups were developed (Group I - 4 °C, Group II - 4.1 to 6.8 °C, and Group III - 6.9 °C). They found that as SSTG was increased (from Group I to Group III), mass activity and non-eosinophilic sperm count were dramatically enhanced, while sperm abnormalities were significantly ($p<0.01$) decreased.

Garcia-Oliveros *et al.* (2020) studied the early sequential effects of heat stress in bull sperm quality Twenty Nellore breed bulls (n=20) were divided into two groups: control and testicular heat stress (scrotal bags/96 h). Sperm motility, abnormalities, plasma membrane integrity, acrosomal membrane integrity, mitochondrial membrane potential, sperm lipid peroxidation, seminal plasma lipid peroxidation, and DNA fragmentation were all assessed in sperm samples. Seven days after heat stress, an increase in sperm abnormalities was found. Sperm lipid peroxidation rose after 14 days, but mitochondrial membrane function, sperm motility, and plasma membrane integrity fell. Effects of heat stress were still present 21 days post scrotal insulation. After 28 days, an increase in sperm DNA fragmentation was identified as a late impact. Heat stress's first impacts (i.e., increased sperm abnormalities and lipid peroxidation) point to the presence of oxidative stress in the sperm, which changes mitochondrial function, sperm motility, plasma membrane integrity, and, later, DNA fragmentation. Heat stress's first impacts (i.e., increased sperm abnormalities and lipid peroxidation) point to the presence

of oxidative stress in the sperm, which changes mitochondrial function, sperm motility, plasma membrane integrity, and, later, DNA fragmentation

Menegassi *et al.* (2018) used infrared thermography to assess the thermoregulation and spermatogenic alterations caused by a scrotal temperature gradient in testicularly compromised bulls (9 Brangus breed bulls 5/8 Angus * 3/8 Nelore). Bulls were insulated for 72 hours (n = 6) and control animals (n = 3) were not insulated at all during the trial. Prior to insult removal, a seminal evaluation was performed, as well as once a week for 13 weeks. In insulated animals, the mean temperature gradient was decreased at the time of insulation removal compared to the week before and after the insult ($p < .05$). Sperm motility was poorer in insulated animals compared to control animals two weeks after treatment ($p < .01$), and spermatozoa total defects were higher in insulated animals ($p < .05$). The primary defects were increased in insulated animals two and seven weeks after insulation compared to control animals ($p < .05$). The temperature gradient in the scrotum was found to have a positive correlation with sperm mass motility. They came to the conclusion that the IRT is a good non-invasive approach for diagnosing thermal insults, especially short-term thermal alterations after removal of scrotal insulation.

2.5 Effect of season on surface temperature of different body Parts

Body temperature of animal increases in environment with high relative humidity and solar radiation (Vermunt and Tranter, 2011) and to cope up with this stressful situation heat is dissipated through different means such as evaporative cooling which are coordinated by hypothalamus (Veissier *et al.*, 2018). Heat from body surface is dissipated through increased peripheral circulation, vasodilatation and sweating (Farooq *et al.*, 2010). Temperature of eye (medial canthus) recorded by IRT reflects the core body temperature in cattle (Gloster *et al.*, 2011). It might be due to its location close to the hypothalamus (thermosensitive site) which reduces lag time in response Stewart *et al.* (2007) and McGreevy *et al.* (2012) reported that eyes (*Caruncula lacrimalis* and posterior border of the eyelids) have superficial capillaries which are highly innervated by the sympathetic nerve fibers. Moreover, eyes have retinal blood vessels similar to the important brain vessels, choroid vessels and ciliary processes, which are similar to the small intestine and kidney. Similar to our study, Sinha (2018) also reported significantly higher body surface temperature during summer as compared to winter.

2.6 Season and its relation with blood/serum hormonal profile

Environmental factors such as radiation, temperature, relative humidity, and wind speed, as well as thermoregulation mechanisms such as conduction, radiation, convection, and evaporation, may exacerbate thermal stress in response to differences in the temperature balance between the animal and the environment. The endocrine system uses chemical messengers called hormones to coordinate and regulate physiological processes (Cunningham, 2008). To counteract the consequences of stress, animals initiate a series of stress-response mechanisms that are regulated by hormones and coordinated by the nervous system and the endocrine system, which is responsible for controlling metabolic, reproductive, and developmental activities (Greenspan, 2006).

The hypothalamo-pituitary-adrenal cortical axis (HPA) and sympatho-adrenal medullary axis (SAM) are activated by heat stress (Minton, 1994), which leads to the release of corticotropin (CRH) by the hypothalamus, which further regulates the release of adrenocorticotrophic hormone (ACTH) by the hypophysis. The ACTH regulates the release of glucocorticoids, including cortisol and catecholamines, adrenalin and noradrenalin by the adrenal (Ferro *et al.*, 2010). Cortisol secretion stimulates the physiological mechanisms which enables the animal to tolerate stress caused by a high ambient temperature (Silanikove, 2000). It is studied that environmental stress that causes an increase in circulating cortisol levels has been shown to influence sperm morphology (Perry *et al.*, 1991).

Heat stress inhibits gonadotropin-releasing hormone (GnRH) in the hypothalamus, which has a direct effect on animal reproductive performance via the hypothalamic-pituitary-gonadal axis. The release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) is inhibited in the anterior hypophysis and due to this gonadotropin secretion in the gonads is altered (River and Rivest, 1991). Hormonal secretion is influenced not only through suppressing HPG, but also by direct action of heat stress on gonadal tissues, resulting in hormonal imbalance and lower sensitivity of cells to FSH and LH action (Santos, 2003). Testosterone is the major androgen that regulates spermatogenesis, the expression of secondary sexual characteristics and sexual behavior (libido) and thus promotes sperm production in bulls (Hafez and Hafez, 2013).

The thyroid gland is one of the most heat-sensitive organs in the body. Thermal exposure has been shown to act directly on the hypothalamic pituitary axis, resulting in a decrease in thyrotropin stimulating hormone and, as a result, decreased in Tetraiodothyronine (T4) and triiodothyronine (T3) hormone concentrations (El Nouty and Hassan, 1983; Fregly, 1989; Reece *et al.*, 2015). Thyroid gland plays the most important role in metabolism, growth and reproduction activity in the body. The metabolic hormone, T4, has been implicated in the physiological regulation of energy balance as well as in maintaining normal reproductive function in mammals (Krishna and Singh, 1998). Thyroid gland has been suggested to have a significant effect on the male reproductive tract, spermatogenesis, and male fertility (Thrun *et al.*, 1997). Thyroid hormones plays an important role in controlling the production of heat in warm-blooded animals and the suppression of the concentration of T3 in response to heat stress, can act as a mechanism for adaptation to reduce thermogenesis. In Khuzestan buffalo bulls' sperm quality and blood serum T3 uptake showed positive relationship in the cold season than in the warm season (Mayahi *et al.*, 2014). Chaudhary *et al.* (2015) also reported significant decrease in T3 level with increase in THI value in Surti buffalo.

Mayahi *et al.* (2014) studied the reproductive performance and thyroid gland functions of buffalo bulls in Khuzestan during the winter and summer seasons. Six male indigenous buffaloes from Khuzestan were used, all of which were of 2-3 years of ages. Semen and blood samples were taken throughout the summer and winter seasons in every two weeks. The concentrations of thyroid hormones and thyrotropin stimulating hormone (TSH) in blood serum were determined using a radioimmunoassay technique. Thyroxin (T4) concentrations were lower in the winter than in the summer ($p \leq 0.05$). T3 uptake was higher in the cold season compared to the hot season ($p \leq 0.05$). There were no significant variations in triiodotyronine (T3) and TSH concentrations, as well as the free thyroxin index Between seasons. Winter had a larger concentration of semen and spermatozoa properties, such as progressive motility, linear velocity, mean velocity, beat cross frequency, linear coefficient, and straightness coefficient, than summer ($p \leq 0.05$).

The effect of season on the endocrinological profile of mithun bulls was investigated by Perumal *et al.* (2017). A total of ten adult mithun bulls, ranging in age from 5 to 6 years old and in good bodily condition, were chosen from the ICAR-NRC on

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Mithun, Jharnapani, Nagaland, India . Based on weather data and sunshine hours, the seasons were classified as winter, spring, summer, and autumn. Blood samples were collected at interval of 4 hours for the whole day and endocrinological profiles were calculated for the hormones viz., FSH, LH, testosterone and thyroxin. FSH, LH, testosterone, and thyroxin were considerably ($P < 0.05$) higher in the spring and winter seasons than in the summer season, while IGF and cortisol were significantly ($P < 0.05$) lower.

The seasonal variation in levels of thyroid hormone and their relation with semen quality and libido in Murrah buffalo bulls was studied by Dixit *et al.* (1984). For study blood samples from 15 Murrah buffalo bulls (10-15 years old) were collected during summer, monsoon and winter seasons and serum was separated. Serum samples were analyzed for triiodothyronine (T3), thyroxine (T4). Total T3 + T4, T3:T4 ratio. T3 and T4 did not reveal any significant differences between seasons.

Rathwa *et al.* (2017) conducted a study to evaluate the impact of summer and winter season on hormonal parameters in Indigenous sheep. 8 adult female sheep of age 2-4 years old with similar physiological status were chosen for experiment. The value of THI during summer and winter seasons were 82.55 and 59.36, respectively, which indicate extreme hot condition during summer and extreme cold condition during winter season. The values of cortisol hormone increased during summer season and values of triiodothyronine (T3), and thyroxine (T4) were decreased significantly during summer season.

Minton *et al.* (1981) studied the level of testosterone hormone in bulls exposed to elevated temperature. 8 Angus bulls were taken, heat stressed (n=4) and control (n=4). The temperature in control chambers was maintained at 22 °C and in treatment chamber 34 °C. Results of study showed that there was slight decrease in testosterone concentration in the bulls exposed to elevated temperature after 15 days of exposure.

Perera *et al.* (1979) observed the seasonal differences in the plasma concentration of testosterone hormone in Murrah buffalo bulls. Blood was collected at intervals of 1 h over a period of 10 h (06.00 to 16.00 h) from each of two adult (3 to 4 year old) Murrah buffalo bulls in April, May, August and December months. The basal concentration of testosterone hormone was below 0.2 ng/ml in bulls during whole sampling periods. Testosterone profile was similar in the two bulls within each sampling period, but

differences were evident between periods. The mean testosterone concentration was highest during august, falling through December to April to reach the lowest value in May.

Ramadan *et al.* (2019) studied whether melatonin administration of buffalo bulls may affect the quality of semen during the nonbreeding season in tropical conditions. 10 Murrah buffalo bulls were randomly allocated into control (n=5) and treated groups (n=5) to study the effect of melatonin treatment on semen characteristics, blood plasma hormonal profile, and antioxidant enzyme activities during nonbreeding season. Melatonin (18 mg/50 kg body weight) was implanted in the treatment group's bulls over a two-month period. Semen was collected twice a week, and blood samples were taken once a week to assess plasma concentrations of melatonin and LH, as well as antioxidant enzyme activity during this period. Melatonin implantation improved sperm qualities during the nonbreeding season by increasing ($P < 0.05$) percentages of sperm with forward motility, viability, total motile sperm, rapid motility, average path, curvilinear, and straight-line velocity and amplitude of lateral head displacement and decreasing ($P < 0.05$) percentages of abnormal sperm when compared to the control group.

Perumal *et al.* (2020) conducted a study to assess the effect of slow release subcutaneous exogenous melatonin (MT) implant on sexual behaviour, scrotal circumference, testicular parameters, endocrinological profile and antioxidant and oxidative stress profiles in mithun bulls during different seasons. Twelve apparently healthy mithun bulls (5-6 years of age) were selected and divided randomly into two groups, group I: Control (n=6) and group II: Treatment (n=6; melatonin implant @ 18mg/50 kg B. Wt). They observed that FSH, LH, testosterone, T4 and melatonin were higher and cortisol and prolactin were lower in spring and winter than in summer season. Concentration of FSH, LH, testosterone and melatonin were higher and concentration of T4, cortisol and prolactin were lower in MT treated than in untreated control group.

Mallick *et al.* (2016) investigated into how the summer and winter seasons affected the quality of sperm and blood plasma hormone levels in cross-bred bulls. Eight adult (3–4 years) Karan fries bulls were selected and blood samples were collected for hormonal analysis. Testosterone concentration was found to be high during summer (7.01 ± 0.92 ng/ml) as compared to winter (3.80 ± 0.31 ng/ml) season. Prolactin concentrations in bulls were found to be 20–40 times higher ($p < 0.001$) in summer (176.53 ± 23.41 ng/ml) months than winter (18.63 ± 2.03 ng/ml) months. Prolactin

showed seasonal change, being greater in summer than winter season. It was concluded that this increased prolactin levels might be involved in meeting increased water and electrolyte demands of the animals in summer season.

Sajjad *et al.* (2007) carried out a study with an aim to determine the blood serum testosterone level and its relationship with scrotal circumference and physical characteristics of semen in Nili-Ravi buffalo bulls. Semen samples were taken weekly for 12 weeks from three 14-year-old buffalo bulls and assessed for physical traits, while blood samples were collected at weekly intervals from each bull and studied for serum testosterone concentrations. There was a positive correlation ($r=0.348$) between testosterone levels and ejaculatory volume. However, there was no association between testosterone levels and sperm motility ($r=0.145$), sperm concentration ($r=0.264$), sperm pH ($r=-0.208$) and total sperm abnormalities ($r=-0.242$).

Perumal and co-workers (2021) designed a study to assess the endocrinological profiles of adult mithun bulls in different seasons (winter, spring, summer and autumn). Follicle stimulating hormone (FSH), testosterone, cortisol, thyroxine (T4), prolactin, and melatonin (MT) were measured at 04:00 h intervals for one entire day in four seasons. The bulls in the winter and spring seasons had significantly more FSH, LH, testosterone, T4 and MT than those in the summer, whereas the bulls in the summer had significantly higher cortisol and prolactin than those in the winter and spring seasons.

2.7 Sexual behavior of bulls and seasonal variation

Sexual behavior is a complicated process that is impacted by the social context, sensory capacity, sexual stimulation, and is regulated by an animal's endocrine constitution (Naskar and Nagpaul, 2005). Libido and mating capability are two parts of sexual behavior (Anderson, 1945). Libido refers to a male animal's willingness and eagerness to mount and perform service of a female (Hultnas, 1959) while the behaviour of the male animal immediately before, during, and after service is known as mating behaviour (Chenoweth, 1981). The degree of sexual expression reflects both the sex desire (libido) and the seminal characteristics of the bull (Joshi and Kharche, 1992). These characteristics are mostly affected by an animal's genetic makeup (Chenoweth, 1983). A breeding bull's libido and mating ability are important features for a successful artificial insemination (AI) programme. The ability of breeding bulls to harvest the highest percentage of spermatozoa in the shortest amount of time is dependent on their

sexual behavior (Amann and Almquist, 1976; Sholikah *et al.*, 2018). The level of sexual arousal and performance influences ejaculation efficiency and semen quality (Pound *et al.*, 2002; Levis and Reicks, 2005; Kondracki *et al.*, 2013). Bulls with a high sex drive have better erections, penile protrusion, ejaculatory thrust, libido, and the ability to mate with females (Singh *et al.*, 2019). Particular emphasis should be given to the surrounding environmental conditions, particularly temperature and humidity, in order to reduce their detrimental impact on semen production and other related physiological functions of breeding bulls (Ahirwar *et al.*, 2018). Male sexual behaviour is influenced by genetic, environmental, nutritional, hormonal, sensory stimuli, age, and sex experience. But the testosterone concentration is greatly affected by the desire to mate or libido (Menegassi *et al.*, 2011). The greatest variations were observed in tropical and sub-tropical areas, where bulls' libido was considerably lowered during the hotter months of the year (Anderson, 1945). In another study by Parmar *et al.* (2019) summer had a negative impact on the sexual behaviour of Jaffrabadi bulls, while winter was the preferred season.

Parmar *et al.* (2019) carried out a study on four mature Jaffrabadi breeding bulls (4–6 years old) to observe the seasonal and bull variations in sexual behavior traits of Jaffrabadi bulls. Experiment was carried out at Cattle Breeding Farm, JAU, Junagadh, for a period of 8 months during winter and summer seasons. Results of the study showed significant differences among seasons for temperament, libido and penile erection score of Jaffrabadi bulls. Libido score (0–9 scale) was found to be 6.75 ± 0.19 during winter and 5.93 ± 0.43 in summer season. It was observed that summer season exerted significant adverse effect on the sexual behavior of Jaffrabadi bulls and winter was the favorable season.

Solanki *et al.* (2019) investigated the effect of season on sexual behavior of Gir bulls. The study was carried out at Cattle Breeding Farm, J.A.U. Junagadh during summer and winter season. Eight Gir bulls were selected and the seasonal variations and correlations amongst the sexual behavior traits were studied. There were significant differences among bulls for sexual behaviour traits in both the seasons. Summer season exerted significantly adverse effect on the sexual behavior of Gir bulls and winter was the favorable season.

Deka *et al.* (2019) studied the sexual behavior traits in 8 Kankrej Bulls (4-5 years old). Semen was collected weekly once for each bull but only fortnightly observations of

semen collections were recorded. Eight collections in a season for each bull were evaluated. Libido score was significantly ($P<0.05$) higher during summer (6.70 ± 0.18) season as compared to winter (5.70 ± 0.19) but libido score during monsoon (6.20 ± 0.23) was non-significant with both summer and winter season.

Ramadan *et al.* (2009) conducted a year-long study on four Egyptian buffalo bulls to examine their sperm characteristics as well as the impact of season on sperm quality and sexual behavior. Bulls employed for routine semen collection were appeared to be in good health and age ranging from three to five years. During the winter, the reaction time was the lowest ($P<0.05$), increased throughout the spring, and maximum during the summer and autumn seasons. Findings of study suggest that increasing THI reduced the libido of bulls (due to a longer reaction time). The study found significant positive correlations ($P<0.01$) between reaction time, ambient temperature, and THI.

2.8 Effect of season on semen quality

Buffalo is a seasonal breeder among dairy animals, with the highest breeding activity in the autumn and winter and the lowest in the summer (Heuer *et al.*, 1987). Changing climate in tropical and subtropical areas has a significant impact on animal productivity and reproduction (Bhakat, 2014). The testicular temperature of should be maintained at about 2-6 °C below their core body temperature for the production of morphologically normal and fertile spermatozoa (Waites, 1970). Summer's hot and humid atmosphere impairs the bull's natural ability to dissipate heat, resulting in heat stress (Menegassi *et al.*, 2015). These elements alters the animal's testicular thermoregulatory mechanism, resulting in an increase in scrotal and testicular temperature as well as a reduction in sperm quality, with a higher percentage of abnormal sperm (Horn *et al.*, 1999; Garcia, 2006; Barros *et al.*, 2015), altered motility and reducing their fertile capacity (Hansen, 2009).

2.8.1 Semen volume

Kumar *et al.* (2020) conducted a study to determine seasonal effects on semen characteristics in Hallikar bulls. For this study 11,008 ejaculates were collected from 25 Hallikar bulls over a period of 15 years. for study each year was divided into four seasons, viz. south-west monsoon (June-September), north-east monsoon (October-December), winter (January- February) and summer (March-May). Results of the study showed that the mean ejaculate volume was 4.67 ± 0.04 , 4.64 ± 0.04 , 4.67 ± 0.03 and

4.65±0.03 ml for winter, summer, south-west monsoon and north-east monsoon season, respectively and these variations in the ejaculate volume among different seasons were found to be non-significant.

Javed *et al.* (2000) carried out a study on Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. Total 16 Nili-Ravi buffalo bulls (*Bubalus bubalis*) were allocated for study. Animals were divided into four age groups, three healthy groups (< 5years, 6-10 years, and >11 year old), and one abnormal group (6-10 year old bulls having poor semen quality). Study period was for a one year and divided into five different seasons (dry summer, humid summer, autumn, winter and spring). Overall semen volume was 4.67±1.62 ml and showed a non-significant difference between age groups, although it was higher ($P<0.05$) in autumn.

Mallick *et al.* (2016) studied the influence of summer and winter seasons on semen quality in Karan Fries bulls. Semen was collected by an artificial vagina method from eight bulls and microscopically evaluated for quality parameters. The semen volume was recorded immediately after collection in graduated semen collection tubes with 0.1 ml calibration. Semen volume was higher in summer (4.83 ± 0.25 ml) season ($p < 0.05$) than winter season (4.07 ± 0.06 ml). They concluded that this increase in volume might be due to increased sexual function through increased photoperiodic stimulation during summer.

Bhakat *et al.* (2011) studied the effect of age and season on semen quality parameters in Sahiwal bulls, maintained at Artificial Breeding Complex, NDRI, Karnal, India. Semen production records of 5,483 ejaculates from 46 Sahiwal bulls since 1996 to 2006 were analyzed for study. It was reported that semen volume (3.87 ± 0.04) and total volume per day (6.09 ± 0.09) was highest during rainy season and lowest during winter (3.72 ± 0.04 and 5.63 ± 0.08 , respectively) season. Significant differences ($P<0.05$) in volume between rainy and winter seasons were observed.

2.8.2 Mass motility

Seasonal influence on various seminal attributes in Murrah buffalo bulls was studied by Bhakat *et al.* (2015) at Artificial Breeding Complex, NDRI, Karnal, from May, 2006 to April, 2007. Data on 156 ejaculates of eight Murrah buffalo bulls of nearly 30 to 58months of age was analyzed during three seasons of year viz., summer, rainy and

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winter. The mean of mass activity (MA) was found to be 2.54 ± 0.70 . Mass activity was maximum during winter (2.67), followed by summer (2.56) and rainy (2.40) season.

A study was conducted by Ram *et al.* (2017) on Murrah buffalo bulls aged between of 5 to 6 years maintained at central semen station, Anjora. They studied the effect of heat stress on seminal characteristics of Murrah buffalo bull semen during winter period (15 °C to 25 °C) and heat stress period (42°C to 48°C). A total of 48 semen ejaculates from 6 Murrah bulls were collected through artificial vagina method and it was observed that the overall mass motility during winter period (3.63 ± 0.09) was significantly ($P < 0.05$) higher than heat stress period (3.33 ± 0.10).

Alavi-Shoushtari *et al.* (2006) carried out study on the seasonal variation in the characteristics of the Azarbaijani buffalo bull semen. Three Azarbaijani water buffaloes bulls of 2-4 years old age kept in the Buffalo Breeding Center, Urmia, northwest of Iran, were selected. Semen samples were collected once a week for a period of one year during Spring, Summer, Autumn and Winter seasons using artificial vagina and a buffalo cow as a dummy. Semen motility (scores) was 3.02 ± 0.06 , 3.02 ± 0.09 , 2.78 ± 0.14 , 2.75 ± 0.135 during spring, summer, autumn and winter seasons respectively. There was a significant variation in the mean values obtained for the percentage of sperm viability it showed an increase in summer and decreased significantly during autumn and winter.

2.8.3 Progressive motility

De Castro *et al.* (2017) conducted a research with the goal of comparing the effect of seasonal temperature variations on ejaculate parameters in Murrah buffalo bulls raised in the humid tropical Amazonian climate. The experiment was divided into two phases: rainy season (RS), which lasted from February to May, and non-rainy season (nRS), which lasted from August to November 2016. For the study, five buffalo bulls were chosen. During the various phases of the experiment, the values of progressive sperm motility decreased. Individual sperm motility was observed to be $80.4 \pm 5.6\%$ percent in the rainy season and $56.2 \pm 13.4\%$ percent in the non-rainy season.

Kumar *et al.* (2020) conducted a study to determine seasonal effects on semen characteristics. For this 11,008 ejaculates collected from 25 Hallikar bulls. The semen characteristics of Hallikar bulls were analyzed for four season i.e. winter, summer, South-West monsoon and North-East monsoon. It was observed that the overall mean initial motility for Hallikar bulls in their study was 80.26 ± 0.11 percent. The mean initial

motility recorded during different seasons for Hallikar bulls was significantly higher during summer $81.60 \pm 0.25\%$ as compared to winter $80.20 \pm 0.33\%$.

Seifi-Jamadi *et al.* (2020) investigated the effect of high ambient temperature during summer on semen quality of Belgian Blue bulls. For this purpose, semen samples were collected from six healthy mature Belgian Blue bulls in March (Low THI group; THI between 30.6 and 56.4) and August 2016 (High THI group; maximum THI of 83.7) respectively. Motility, morphology, acrosome integrity, chromatin condensation, viability, and reactive oxygen species production were assessed for frozen-thawed semen. The frozen-thawed low THI spermatozoa had higher total motility (52.36 ± 1.31) compared to high THI group (42.23 ± 1.26).

Valeanu *et al.* (2014) conducted a study to see how seasonal variables such as temperature, atmospheric pressure, and day length can affect the quality of dairy bull sperm. Ten Swedish Red and White (SRB) dairy bulls, ranging in age from four to seven years, were used to collect semen samples. Cryopreserved semen straws with an acceptable post-thaw quality were included in this study. From spring (April–May), summer (July–August), and winter (December–January) collections, one straw of frozen semen was chosen for each bull. Progressive motility (%) was 51.01, 49.37 and 48.38 during winter, spring and summer seasons respectively.

Hameed *et al.* (2017) investigated the effect of season and climatic variables on the semen parameters of Nili-Ravi buffalo breeding bulls in the Sahiwal and Bahawalpur districts of Punjab, Pakistan. 14 adult Nili-Ravi buffalo breeding bulls were employed in this research ($n=7$ from each of the Semen Production Units (SPU) Sahiwal and Bahawalpur). The research was carried out over the course of a year, divided into four different seasons: autumn, winter, spring, and summer. Summer was divided into two categories: Dry Summer and Humid Summer. Semen samples were collected fortnightly and two ejaculates were taken from each bull, which were evaluated for semen quality. Individual sperm motility (percent) during Autumn, Winter, Spring, Dry Summer, and Humid Summer was 67.41 ± 4.51 , 61.17 ± 4.51 , 70.21 ± 4.51 , 66.16 ± 4.51 and 65.12 ± 4.51 in Sahiwal district and 53.13 ± 4.5 , 45.00 ± 4.51 , 49.93 ± 4.51 , 54.47 ± 4.51 and 46.07 ± 4.51 in Bahawalpur district, respectively. In the Sahiwal district, seasonal effects on individual sperm motility were found to be non-significant ($P > 0.05$). Individual sperm motility was lowest during the winter season and highest during the spring season. Individual sperm motility in buffalo bulls from Bahawalpur district was

also non-significant ($P>0.05$) in different seasons, with the highest individual sperm motility in dry summer, followed by autumn, spring, humid summer, and winter seasons in that order.

Tuli and Singh (1983) studied the effect of season on freezability of buffalo semen. 56 semen ejaculates from seven Murrah buffalo bulls with mass motility >3 (based on 5 point scale) were frozen during winter and summer season. The average pre and post freezing (after 30 days) motility during summer and winter season was 64.64 ± 1.37 , 34.82 ± 1.07 and 66.25 ± 1.14 , 42.32 ± 1.29 respectively. The loss in motility of spermatozoa frozen during summer season was due to its comparatively poor vitality during summer season and it was concluded that for better sperm motility semen should be frozen during winter season.

2.8.4 Sperm concentration

Kumar and Sharma (2018) studied a total of 120 ejaculates collected from ten Bhadawari buffalo bulls to observe the effect of Month on Semen quality and freeze ability during frozen semen production. The results of the study showed that the average sperm concentration of all Bhadawari bulls was 0.95 ± 0.02 million/ mm^3 and it showed Significant ($P<0.05$) effect of months on sperm concentration. Highest sperm concentration (1.02 ± 0.04 million/ mm^3) was recorded in the month of December.

The effect of season on semen quality in seven Murrah buffalo bulls was studied from January 2002 to December 2005 by Tiwari *et al.* (2011) at Animal Breeding Center, Salon. As per climatic data collected at the center seasons were classified into winter, summer and monsoon. The mean sperm concentration observed was 1139.71 ± 5.89 million/ml. Sperm concentration differed significantly ($P<0.05$) between bulls as well as between seasons. Difference in sperm concentration during the summer (1159.01 million/ml) and monsoon (1147.15 million/ml) season was not significant, but it was significantly lower during winter season (1111.86 million/ml).

Sharma *et al.* (2018) studied seasonal variation in semen characteristics of buffalo bulls to quantify the effect of temperature humidity index (THI) on various semen quality parameters. A total of 42 ejaculates from 2 buffalo bulls were collected. Semen collection was done twice a week through AV (artificial vagina) method for 6 months. The THI of the study period was divided into 3 groups, i.e. Group I (64 to 72, $n=10$), Group II (72 to 78, $n=13$) and group III (78 to 84, $n=18$) and it was observed that

THI variations had significant effect on concentration of sperm. The results of this study indicated that the concentration of sperms decreased significantly ($P<0.05$) with the increase in THI and it was concluded that the hot-humid season with Higher THI adversely affect the various seminal attributes of buffalo bull.

Isnaini *et al.* (2019) carried out a study to evaluate the seasonal variation on semen characteristics of Murrah buffalo bulls in Indonesia. A total of three Murrah buffalo bulls were included in this study. The semen was collected once a week during the rainy season (from January to April and from October to December) and the dry season (from May to September). Immediately after collection, the semen quality was evaluated and it was observed that the sperm concentration, total sperm output, and straw production were decreased in the dry season (0.91 ± 0.03 billion/mL, 3.89 ± 0.1 billion, 151.13 ± 5.43 unit/collection, respectively) ($P<0.05$) as compared to the rainy season (1.22 ± 0.03 billion/mL, 5.20 ± 0.23 billion, 209.54 ± 9.04 unit/collection, respectively).

2.8.5 Sperm viability

Nitharwal *et al.* (2017) investigated the influence of season on frozen semen quality in 6 Murrah buffalo breeding bulls, semen samples of bulls were collected and frozen in 4 different seasons, viz. summer (May-Jun), rainy (Jul-Aug), winter (Dec-Feb) and spring (mid Feb-Apr) The mean percent sperm viability in Murrah buffalo bulls during different season was 66.54 ± 1.90 , 55.69 ± 1.79 , 49.00 ± 3.82 and 52.24 ± 1.82 during winter, spring, summer and rainy season, respectively. Sperm viability showed significant ($P<0.01$) variation among the seasons and higher per cent sperm viability was obtained in winter as compared to other seasons.

Alam *et al.* (2015) studied the effect of seasonal on semen quality of Egyptian water buffalo (*Bubalus bubalis*) bulls. The semen quality was evaluated by studying sperm motility, plasma membrane integrity, viability, morphology and level of lipid peroxidation (Thiobarbituric acid reactive substances; TBARS), DNA integrity and fertilizing ability of semen samples collected in winter and summer seasons. Results indicated that sperm viability of winter ($78.8\pm 17.84\%$) samples was significantly higher than that of summer ($72.42\pm 14.66\%$) samples.

Koonjaenak *et al.* (2007) examined whether seasonality affects post-thaw viability and plasma membrane integrity in 18 Thai swamp buffalo sires. Frozen semen doses, prepared between 2003 to 2005 and 1980 to 1989 and from 18 AI Thai swamp

buffalo sires were used for analysis. All the semen quality parameters were compared over 3 seasons of the year (summer: March–June, rainy: July–October and winter; November–February), with different ambient temperature and humidity. The percentage of spermatozoa with intact plasma membrane (live, SYBR-14+/PI₋) was significantly ($P < 0.001$) higher in winter (54.6%) than in the summer (46.7%) or rainy season (43.5%) seasons.

A study was undertaken at Basra University in Iraq by AL-Asadi *et al.* (2021). To evaluate the relationship between parameters such as age, seasons, and the attributes of semen in Jonobi bull from 11/2/2017 to 2/1/2018. The study used a total of 12 Jonobi bulls (ages 2-4 years old). During the Spring, Summer, Autumn, and Winter seasons, values of live spermatozoa (percent) for bulls of 4 years old were 77.36, 84.93, 87.77, and 80.97, respectively. As compared to bulls of different ages, 4 year old bulls had a substantial rise ($P < 0.05$) in the proportion of live sperm and a significant ($P < 0.05$) decrease in the percentage of dead sperm in the semen.

Das *et al.* (2017) investigated seasonal fluctuation in the properties of northeast India's swamp buffalo bull semen. Based on meteorological data, the season was classified into four categories: pre-monsoon (March to May), monsoon (June to September), post-monsoon (October to November), and winter (December to January). The study included data from four bulls (four years old) with different semen qualities. During the pre-monsoon, monsoon, post-monsoon, and winter seasons, livability (%) was 89.97 ± 0.77 , 89.36 ± 0.85 , 90.21 ± 0.80 , 90.10 ± 0.71 , respectively. With an average mean of 89.910.19, the percentage of viable sperm ranged from 89.36 ± 0.85 to 90.21 ± 0.80 . The highest value was reported during the post-monsoon period, although it was not statistically significant.

2.8.6 Sperm morphology

Koonjaenak *et al.* (2007) investigated seasonal variation in sperm quality in five mature, healthy swamp buffalo bulls (*Bubalus bubalis*) in Thailand over three seasons (rainy, i.e. July–October; winter, i.e. November–February and summer, i.e. March–June). The overall total mean percentages of sperm abnormalities in buffalo bull spermatozoa were $< 15\%$, with the rainy season accounting for $(13.7 \pm 0.5\%)$, winter accounting for $(12.4 \pm 0.5\%)$, and summer accounting for $(10.7 \pm 0.5\%)$ percent. Summer had the highest proportion of morphologically normal spermatozoa and winter had the lowest

($P < 0.05$). Only the proportion of tail defects was affected by season, with the proportion being highest in the rainy season and lowest in the summer.

De Castro *et al.* (2017) conducted a study with the objective to compare the influence of seasonal temperature variations on parameters of semen ejaculates of Murrah buffalo bulls, raised under the humid tropical Amazonian climate. Five buffalo bulls were selected for the study. The experimental period was divided into two phases: non rainy season (nRS), from August to November and rainy season (RS), from February to May. Collection of ejaculates was done twice a week. A total 30 samples were collected per bull, namely 10 ejaculates in the RS and 20 ejaculates in the nRS. The evaluation of the semen samples was performed on fresh semen, immediately after collection $2 \pm 3.9\%$, total defects $20.2 \pm 5.3\%$ whereas in the nRS, major defects were $20.8 \pm 9.9\%$, minor defects were $27.5 \pm 6.3\%$, total defects were $48.3 \pm 9.3\%$. It was concluded that the ideal period for collection of buffalo ejaculates were the months of the rainy season in this period a better quality ejaculate of buffaloes were obtained.

Bhakat *et al.* (2015) studied the influence of season on semen quality in 8 Murrah buffalo bulls. Year of collection divided into three seasons: summer (April to June); Rainy (July to October) and winter (November to March). Sperm abnormalities head (2.79%, 2.68% and 1.53%), midpiece (2.15%, 1.58% and 1.1%) and tail (7.11%, 5.61% and 4.01%) were during summer, rainy and winter season respectively. They reported the significant ($P < 0.01$) variation in head, mid-piece, tail and total abnormality percent among the seasons and also between season. All the abnormalities were found to be higher during summer season followed by rainy and winter seasons.

A study based on the effect of seasonal variations on sperm morphology was conducted by Singh *et al.* (2018). Study was conducted on four, adult, fertile and healthy Harijana bulls, aged between 5.5-6.5 years. The study was conducted over a period of four months and divided into two seasons as winter season (December- January) and summer season (May-June). 24 semen samples were collected in each season (six ejaculates from each bull) by using Artificial Vagina. They found that Head abnormalities (%) 0.79 ± 0.10 and 1.21 ± 0.15 ; Mid-piece (%) 0.50 ± 0.12 and 0.67 ± 0.13 ; Tail (%) 1.42 ± 0.12 and 2.21 ± 0.17 ; Total abnormalities (%) 2.71 ± 0.19 and 4 ± 0.33 during winter and summer season respectively. Total morphological abnormality in Harijana bull spermatozoa was higher in summer season in comparison to winter. And it was found that winter was the most favourable season for good quality semen production.

Ramadan *et al.* (2009) conducted a 12-month study on four Egyptian buffalo bulls to determine the characteristics of their semen and the impact of seasonal and climatic variables on the quality of their sperm. Bulls selected for routine semen collection were appeared to be healthy and ranging in age from three to five years. Abnormal sperm (%) was 9.41 ± 0.09 , 12.7 ± 0.8 , 15.9 ± 1.11 and 8.66 ± 1.05 during winter, spring summer and autumn season. In the study, abnormal spermatozoa showed significant seasonal variations ($P < 0.01$). During the spring and summer seasons, the proportion of aberrant sperm was substantially higher ($P < 0.05$).

Seasonal variation in semen quality in *Bos indicus* and *Bos taurus* bulls bred in tropical circumstances was investigated by Nichi *et al.* (2006). In this study sixteen Simmental and eleven Nelore bulls age between three to four years were selected. Semen samples were collected consecutively for 2 years during summer and winter season. Simmental bulls showed significantly greater percentages of major sperm abnormalities in the summer than in the winter, ($20.3 \pm 3.1\%$ versus $12.2 \pm 2.4\%$, respectively) and they had much higher percentages of major defects in the summer than Nelore bulls ($20.3 \pm 3.1\%$ versus $10.1 \pm 2.9\%$). The rise in the total number of abnormal sperm all across the summer corresponds to the well-known impact of rising temperatures on sperm quality.

2.8.7 Sperm acrosome damage

Nitharwal *et al.* (2017) observed the effect of season on semen quality in Murrah buffalo bulls during different seasons of a year. Frozen semen samples of 6 Murrah buffalo bulls were assessed for sperm acrosome damaged and it was $26.71 \pm 3.06\%$, $32.06 \pm 5.04\%$, $31.26 \pm 0.31\%$ and $31.93 \pm 4.62\%$ during winter, spring, summer and rainy season, respectively. Sperm acrosome membrane damage was lower in winter as compared to other seasons.

Parmar *et al.* (2019) carried out a study on semen 192 ejaculates collected from four Jaffarabadi breeding bulls. Samples were collected by artificial vagina method in morning hours and Cryopreserved at Frozen Semen lab of Cattle Breeding Farm, JAU, Junagadh, Gujarat from November 2017 to October 2018. The percent post thaw acrosome integrity of spermatozoa during winter and summer seasons were 75.22 ± 0.27 and 74.29 ± 0.22 respectively. Statistically it was non-significantly higher during winter as compared to that of the summer season.

Baruti *et al.* (2019) carried out a study on 15 mature, healthy swamp buffalo bulls aged 5–8 years that were kept at the College of Veterinary Science in Khanapara, Guwahati. Seminal characteristics were studied during three seasons of the year (Summer, Monsoon, Autumn and winter) each with a different ambient temperature and humidity. Acrosomal Integrity (%) was 51.64 ± 0.35 , 54.57 ± 0.27 , 58.01 ± 0.63 and 59.46 ± 0.36 during summer, Monsoon, autumn and winter season respectively.

Sinha (2018) studied the effect of season on semen quality and fertility of Murrah buffalo bulls. Study was carried out on eight Murrah buffalo breeding bulls. Experimental period was divided into four seasons (winter, summer, rainy and autumn season). Various semen quality parameters such as volume, mass activity, concentration, HOST, acrosome integrity and abnormalities were studied. Acrosome integrity was significantly lower in both summer (86.96 ± 0.69) and rainy (88.33 ± 0.56) seasons as compared to winter (92.71 ± 0.62) and autumn (91.21 ± 0.69) season.

Soren *et al.* (2016) studied the influence of season on semen quality in Karan Fries (Tharparkar \times Holstein Friesian) bulls. Study was conducted on five ($n=5$) Karan Fries bulls (4-6 years of age) from Animal Breeding Research Centre (ABRC) of National Dairy Research Institute (NDRI), Karnal. Weekly ejaculates were collected during winter (December to mid-February), spring (mid-February to April), hot dry (May to June) and hot humid (July to August) seasons. Semen samples were collected using artificial vagina ($42-45^{\circ}\text{C}$) at early in the morning and immediately after collection semen evaluation was carried out for parameters like, volume, mass motility, individual motility, hypo-osmotic swelling test (HOST), non-eosinophilic sperm count, acrosomal integrity, sperm concentration and abnormalities. Acrosomal integrity (%) was 74.22 ± 0.62 in hot humid and 83.40 ± 0.48 in spring season it showed a significant ($p < 0.05$) difference between hot humid and spring season.

2.8.8 Hypo osmotic swelling test

Bhakat *et al.* (2011) collected one hundred and fifty six ejaculates from eight Karan Fries (KF) bull to study the effect of season (Summer, $N=42$; Rainy, $N=51$ and Winter $N=63$) on semen quality parameters at ABRC-NDRI, Karnal, India. They reported highly significant seasonal variation in hypo-osmotic swelling test. HOST reacted spermatozoa observed in the present investigation was maximum during winter 50.00% season followed by rainy 36.44% and summer 36.41% season.

Review of Literature

Baruti *et al.* (2019) carried out a study on fifteen mature, healthy swamp buffalo bulls of age 5–8 years maintained at College of Veterinary Science, Khanapara, Guwahati. Seminal characteristics were compared between three seasons of the year (Summer, March–June; Monsoon, July–September; Autumn, October–November and winter, November–February) with distinct ambient temperature and humidity. HOST (%) was 68.65 ± 0.54 , 70.45 ± 0.63 , 72.79 ± 0.87 , 76.86 ± 0.34 during summer, Monsoon, Autumn and Winter seasons respectively. HOST reacted spermatozoa observed in the present investigation was maximum during winter season than the summer season

Khawaskar *et al.* (2012) carried out a study at the Semen Station of Veterinary College, AAU, Anand, Gujarat. According to the prevailing agroclimatic conditions of the region, the year was split into three seasons (monsoon, winter, and summer). They collected 60 semen ejaculates (10 ejaculates/bull/season) from two sexually mature and healthy Surti buffalo bulls. Individual sperm motility, sperm concentration, live sperm, total abnormal sperm, and sperm with intact acrosome were all recorded. During the monsoon, winter, and summer seasons, hypo osmotic resistant sperm (%) was 56.35 ± 3.62 , 65.62 ± 4.27 and 55.87 ± 4.90 respectively. Results of the study proved that, winter is the best time for Surti buffalo bulls to produce spermatozoa with a higher proportion of intact plasma membrane.

Mandal *et al.* (2003) investigated the motion characteristics of Murrah buffalo bull spermatozoa in different seasons and their association with plasma membrane functional integrity. Six adult Murrah buffalo bulls (3.5–7 years old) were used in study and semen samples were collected at weekly intervals using an artificial vagina. Routine quality parameters were evaluated immediately after semen collection. HOS-reactive spermatozoa revealed considerable seasonal variation. During the winter season, the HOS reactivity (%) of spermatozoa was highest (65.04 ± 0.05). The percentage of HOS-positive spermatozoa did not differ significantly between the summer (54.5 ± 0.06) and rainy (55.4 ± 0.05) seasons.

CHAPTER –3

Materials & Methods

MATERIALS AND METHODS

3.1 Location of the study

The present investigation was carried out at Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal (Haryana). The institute is located at an altitude of 250 meters above the mean sea level on 29.43°N latitude and 72.2°E longitude. The maximum ambient temperature goes up to 45°C during summer and minimum about 2°C during winter. The annual rainfall is about 760 to 960 mm, most of which is received during July and August. Relative humidity ranges from 41 to 85 per cent. (www.weather-forecast.com/locations/Karnal).

3.2 Experimental animals

For this study 14 mature Murrah buffalo bulls of around 3-4 years age were selected for the study by taking their body condition and health status. Animals were maintained at Artificial Breeding Research Centre of the institute. Bulls selected for the experiment were healthy, free from diseases, sexually mature, in good libido condition and clinically normal. The bulls were kept under identical feeding and management conditions during the entire course of investigation. Semen was collected in the morning hours from the bulls twice a week with two ejaculates on the same day with 15 to 30 minutes rest between successive ejaculates. All the experimental procedures and animal experimentation methods were approved by the Institutional Animal Ethics Committee.

3.3 Duration of experiment

Experiment was conducted from December 2020 to June 2021 covering winter, spring and summer seasons.

3.4 Housing and management of bulls

Bulls were kept in individual bull pen (30'×10') under loose housing system on concrete floor with the orientation of east-west direction along its long axis. The pens were separated by solid partitions that restricted both direct physical and visual contact of bulls in adjacent pens. One third of the area was covered with corrugated asbestos sheets and rest was open. The sheds' were cleaned once daily early in the morning. The bulls were fed concentrate ration (with 21% CP and 70% TDN) as per ICAR feeding standard at 9.00 A.M. daily and seasonal chaffed green fodder like maize, barseem,

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jowar and oats, etc depending on their availability was fed ad lib to the animals. Bulls were having free access to clean drinking water round the clock. They were given exercise for one hour the day prior to semen collection in the rotatory bull exerciser so as to maintain the physical health and sexual vigour of the bulls and to ensure quality semen production. Vaccination, deworming and other herd-health program were followed as per the farm schedule to ensure good health. The bulls were thoroughly washed and groomed at least half an hour before semen collection.

3.5 Cleaning and sterilization of equipment and glassware

Rubber wares, artificial vagina (AV), buffer solutions, plastic tips, filter papers and distilled water other such materials were sterilized by autoclaving at 15 lbs pressure at 121°C for 20-30 minutes. After sterilization, the AV was stored for overnight in an incubator at 45°C. After autoclaving buffer was cooled and kept in the refrigerator. The stains, polyvinyl alcohol and other polyethene articles were exposed to ultraviolet rays for one hour before use.

All the glassware were washed and cleaned with running tap water and soaked in warm neutral detergent for at least 30 minutes. Thereafter, the items were thoroughly cleaned under running tap water using a brush. The materials were rinsed thoroughly with double distilled water to completely remove detergent residue and other impurities. After cleaning, the glass wares were dried by keeping them inverted on blotting paper. Dry glassware was wrapped in aluminum foil and then covered in clean paper before being sterilized in a hot air oven at 160°C for 1 hour.

3.6 Objective 1: To study the effect of season on physiological responses and hormonal profile in Murrah buffalo bulls

Experiment was conducted from December 2020 to June 2021, covering winter, spring and summer season.

Table 3.1: Classification of season

Sr. No.	Season	Duration
1	Winter	15 th December to 15 th February
2	Spring	16 th February to 30 th April
3	Summer (hot dry)	1 st May to 30 th June

3.6.1 Recording of meteorological data

The data regarding climatic variables like temperature, relative humidity during the study period was taken from Central Soil Salinity Research Institute (CSSRI), Karnal, India. Temperature humidity index (THI) was calculated with the help of aforementioned variables as described by West, 1994.

$$\text{Temperature humidity index (THI)} = \text{td} - (0.55 - 0.55\text{RH}) (\text{td} - 58)$$

Where, td = dry bulb temperature

RH is expressed as a decimal

3.6.2 Recording of physiological parameters

- **Rectal temperature (°C):** rectal temperature was recorded at weekly interval using digital thermometer by inserting it in the rectum and touching the bulb to rectal wall for 2 minute duration in the morning (6 to 7 AM) and afternoon (2 to 3 PM) hours.
- **Respiration rate (Breath/min):** Respiration rate was recorded at weekly interval on the same day of temperature recording in the morning and afternoon hours by counting the movement of flank, i.e., one inward and one outward movement was counted as one respiration.

3.6.3 Infrared thermography of body parts and scrotal surface

The temperature of different body parts such as forehead, eye, flank and rump and scrotal surface of male was taken by the digital infrared thermography camera by using a hand-held high resolution digital thermal imaging FLIR i7 camera from a distance of 1 meter. Before taking images the body surface and scrotal area was made free from dung and manure. The thermal profile of the scrotum was measured by positioning the camera perpendicular to the scrotum. At three different points of the scrotum i.e. upper surface/proximal pole temperature (PPT), mid surface/ mid pole temperature (MPT) and lower surface/distal pole temperature (DPT) were recorded for estimation of scrotal surface temperature (Menegassi *et al.*, 2015). Scrotal surface temperature gradient was calculated as difference between PPT and DPT. IRT images of best quality in terms of focus and resolution were selected for analysis. A constant area of sharp and focused IR images of particular points were selected, interpreted and analyzed by the FLIR tools analysis software and an average temperature of each point

was recorded. The thermal imaging of body surfaces was recorded at interval of 15 days across the seasons.

3.6.4 Hormonal estimation

3.6.4.1 Collection of sample and methodology

For hormonal estimation (T3, T4, TSH, Cortisol and Testosterone) blood samples of bulls were collected from the jugular vein in vacutainer tubes on the day of semen collection. Immediately after blood collection, the tubes were transported to the laboratory in an icebox for further processing. The blood samples were kept for 1 hour without disturbance and then centrifuged to obtain serum which was aliquoted in different fractions and stored at $-20\text{ }^{\circ}\text{C}$ till estimation of hormones. Serum samples were analyzed by Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of hormones with the help of auto analyzer.

3.6.4.2 Biological principle of the procedure for analysis of T4

Total T4 assay is a two-step immunoassay to determine the presence of thyroxine (Total T4) in serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology. In the first step, sample and anti-T4 coated paramagnetic microparticles are combined. Bound T4 is removed from the binding sites on thyroxine binding globulin, pre-albumin and albumin. T4 present in the sample binds to the anti-T4 coated microparticles. After washing, T3 acridinium-labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of Total T4 in the sample and the RLUs detected by the optical system.

3.6.4.3 Biological principle of the procedure for analysis of T3

Similar to the procedure followed for T4

3.6.4.4 Biological principle of the procedure for analysis of TSH

This is a two-step immunoassay to determine the presence of thyroid stimulating hormone (TSH) in serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology. In the first step, sample, anti- β TSH antibody coated paramagnetic microparticles and TSH Assay Diluent are combined. TSH present in the sample binds to the anti-TSH antibody coated microparticles. After washing, anti- α TSH

acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured by the optical System

3.6.4.5 Biological principle of the procedure for analysis of Testosterone

A competitive immunoassay technique is used, which depends on competition between testosterone present in the sample with a horseradish peroxidase (HRP)-labeled testosterone conjugate for a limited number of binding sites on a biotinylated antibody (mouse anti-testosterone). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of testosterone present.

3.6.4.6. Biological principle of the procedure for analysis of cortisol

A competitive immunoassay technique is used, which depends on a competition between cortisol present in the sample with a horseradish peroxidase (HRP)-labeled cortisol conjugate for a limited number of binding sites on a biotinylated antibody (sheep anti-cortisol). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system.

3.7 Objective 2: To investigate the effect of different season on sexual behavior and semen quality in Murrah buffalo bulls.

3.7.1 Sexual behavior of Murrah buffalo bulls

Sexual behavior was recorded at the time of semen collection. Dummy bulls were used for semen collection of Murrah buffalo bulls. Different dummy bulls were used on

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different days to minimize sexual satiation of bull from same dummy, to provide uniform stimulus pressure and randomize dummy effect. Semen was collected twice a week semen collection schedule (two ejaculates in one collection at an interval of 15-30 minutes). Each bull was assigned to be handled by two experienced handlers who were familiar with the bulls. On the day of semen collection bulls were taken to the collection yard, where two bulls were kept as dummy. Bulls were led to a dummy and freely permitted to mount and service an artificial vagina. Each animal were sexually stimulated and two false mounts were given before semen collection. The sexual behaviour scoring was adapted as described by Anzar *et al.* (1993).

✚ Reaction Time (Sec.) - It is the time taken by buffalo bull from exposure to dummy until its mounting.

✚ Sexual aggressiveness (SA) – It is the behavior of a bull during approach towards the teaser. It was assessed visually, and the bulls were classified as:

1. Aggressive- uncontrollable, extremely eager to mount and approached teaser with full vigor.
2. Active- approached teaser with less vigour and aggression
3. Dull- proceeded with a dull expression and took a longer time to mount than their counterparts.
4. Shy- exhibited mild sexual interest and was reluctant to mount.

Table 3.2: Reaction time and Sexual aggressiveness score for bulls

RT (sec)	score	SA	Score
<5	6	Aggressive	4
6-15	5	Active	3
16-30	4	Dull	2
31-60	3	Shy	1
61-120	2		
121-300	1		
>300	0 (Refusal to mount)		

Tactile stimulations (TS) - after approaching the teaser buffalo bulls exhibited certain behavioral characteristics these are called tactile stimulations. It consists of sniffing, bunting, licking, chin-resting, flehmen reaction, licking of penis and urination, etc.

Libido Score is calculated using the formula

$$\text{Libido score} = \{[(\text{RT Score} + \text{SA Score}) - 0.2 \text{ per TS}] / 10\} \times 100$$

RT – reaction time

SA – Sexual aggressiveness

TS – Tactile stimulation

For each tactile stimulation expression 0.2 was deducted from the total score obtained for reaction time and sexual aggressiveness.

If a bull did not mount on the first attempt, then the dummy was changed. If bull did not mount the second dummy in the prescribed time (5 min) then a refusal to mount designation was noted and a 0 score was given. Reaction time and sexual aggressiveness of only successful attempt was used for libido scoring.

- False mount – it is an effective way to sexually stimulate the bulls. Generally 2-3 false mounts are given to maximize the sperm count per ml of semen.
- Total time taken to ejaculate (sec.) – it is the duration of time taken by a non-stimulus bull from appearance to mount with successful ejaculation when it is brought to a stimulus bull (Elrabie *et al.*, 2008).

3.7.2 Semen collection and evaluation

Semen was collected twice a week using bull specific Artificial Vagina (IMV Technologies, France) method, with standard semen collection procedure. Semen collection was performed by well trained and experienced persons at Artificial Breeding Research Center, NDRI, Karnal. Semen was collected in the morning hours beginning at 7:30 AM. Before collection bulls were thoroughly cleaned and allowed to dry for 15-20 min. The temperature of AV was maintained between 42 to 45° C with sufficient pressure. The bulls were sexually prepared by given at least two false mounts before allowing serving. Two successive ejaculates were taken from each collection at an interval of at least 15 to 30 minutes. During collection, AV was placed with the

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horizontal plane of the dummy. During mounting, the penis was quickly guided into the artificial vagina. Immediately after semen collection, collecting tubes containing semen was passed to the laboratory for quality evaluation and further processing. Immediately after collection, the ejaculates were brought in the laboratory and placed in water bath maintained at 32°C for assessing volume, color, mass-activity, individual motility, sperm concentration and subsequent processing .

3.7.2.1 Ejaculate Volume

The volume of semen ejaculates was measured directly from the sterilized graduated glass tube (15 mL) having 0.1 mL calibration.

3.7.2.2 Mass activity

Mass activity of freshly ejaculated sperms was assessed as per method described by Tomar *et al.* (1966). A drop of neat semen was placed on clean grease free glass slide without applying cover slip mounted on a thermal stage maintained at 37°C and examined under phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) at 10X. Mass activity score was noted on the basis of swirls activity of semen. The presence of waves and eddies throughout the whole drop was observed and on the basis of the intensity of waves and eddies, the ejaculates were graded on a numerical scale of 0 to 5. The corresponding activity and the score are as tabulated:

Table 3.3: Mass activity score of semen

Sr. No.	Semen Activity Characteristics	Motility Score (0 to +5)
1.	Rapid waves and swirls	+5
2.	Less rapid swirls and eddies	+4
3.	Swirls are slowly scattered in the field	+3
4.	Swirls absent and individual movement of spermatozoa are more evident from the field	+2
5.	No wave motion observed	+1
6.	Spermatozoa are immobile, no motion	0

3.7.2.3 Individual sperm motility

The motility was recorded as a percentage of progressively motile spermatozoa in the extended semen. This was assessed by placing a drop (6-8 μ L) of diluted semen (diluted with Tris egg yolk extender) on a clean, grease-free glass slide mounted on a warm stage maintained at 37°C and observed under phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) at 20X objective after covering with a cover slip. Sperms in the slide were examined in at least five randomly chosen microscopic areas. Percent progressive motility, under a scale of 0-100%, was calculated by taking the mean of progressively motile sperms in all areas.

3.7.2.4 Sperm concentration

The concentration of spermatozoa (in millions/mL) in the fresh semen was determined by a photometer (IMV, L'Aigle, France). Bovine photometer used 40 μ L of semen diluted with 3960 μ L of NaCl. It directly gives the values for concentration of spermatozoa.

3.7.2.5 Sperm viability

The viability of fresh semen samples was assessed by staining technique using Eosin-Nigrosin stain (Campbell *et al.*, 1953). Eosin-Nigrosin stain used to determine live and dead sperm count.

Preparation of stain and staining method

- For preparation of stain 5 g of Eosin-Y (water soluble) and 10 g of Nigrosin are dissolved separately in 100 ml of 2.9% sodium citrate solution.
- Both the mixture is then boiled for 15 min. separately and the amount of volume which got evaporated during boiling is replaced with the buffer.
- The mixture was filtered through good quality filter paper (Whatman filter paper no. 40) and stored in a dark and sealed glass bottle.
- Finally stain is prepared by mixing 10% Nigrosin solution and 5% Eosin-Y solution in the ratio of 4:1 in a container.
- For staining one drop of semen sample is mixed with three drops of stain and the semen-stain mixture is allowed to rest for about 1 min. After 1 min, a thin smear is prepared on a clean, grease free microscopic slide (pre-warmed to 37°C), air

Materials and Methods

dried and then observed at 100× magnification oil immersion objectives of phase contrast microscope.

- The sperm which appears colorless or white are considered as live and those appeared partially or completely pink colored are considered as dead. A total of 200 spermatozoa are counted in each slide and percentage of live and dead sperm is determined.

3.7.2.6 Sperm acrosome integrity

Acrosome integrity was evaluated by Giemsa staining method described by Hancock (1952) and Watson (1975). Percentage of the intact acrosome was calculated as number of acrosome stained spermatozoa to the total number of sperms counted.

Preparation of stains and solutions

A.) Preparation of Giemsa Stock solution

Table 3.4: Composition of Giemsa stain (stock solution)

Sr. No.	Chemical	Quantity
1	Giemsa stain	3.8 g
2	Absolute alcohol (GR grade)	375 mL
3	Glycerol (AR grade)	125 mL

Giemsa stain was grounded with absolute methanol in a pestle and mortar. To this glycerol was added. Stain mixture was stored at 37°C for one week. During this storage period, it was shaken for few minutes each day. After 7 days the stain was filtered through Whatman filter paper No. 40 and stored in a dark and sealed glass bottle for further use.

B. Preparation of fixative

Table 3.5: Fixative for Giemsa staining

Sr. No.	Chemical	Quantity
1	NaCl	8.5 g
2	NaH ₂ PO ₄ .H ₂ O	4.0 g
3	NaH ₂ PO ₄	6.5 g
4	40 % Formaldehyde	100 mL
5	Distilled water	900 mL

10% formalin was used for fixation of spermatozoa

C.) Preparation of Sorenson's phosphate buffer**Table 3.6: Composition of Sorenson's phosphate buffer**

Chemicals		Quantity
Solution A	Sodium phosphate dibasic ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$)	11.876 g
	Distilled water	1000 mL
Solution B	Potassium phosphate monobasic ($\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$)	9.08 g
	Distilled water	1000 mL

0.1 M Sorenson phosphate buffer was prepared by adding 33 mL of solution A and 17 mL of solution B

D.) Preparation of working Giemsa Solution

On the day of experiment Giemsa Stock solution was diluted to final concentration of 10% (3 mL) in 0.1 M Sorenson's buffer (2 mL) and 35 mL triple distilled water and makes final volume of 40 mL in a coplin jar.

Staining procedure

A thin smear of extended semen was prepared on a clean, grease-free dry slide. The smear was air-dried at room temperature for at least 10 minutes. The smear was fixed by immersing in fixative solution for 25 -30 minutes in a coplin jar. Then it was washed in running tap water then with distilled and air dried. Again the slide was immersed in coplin jar having buffered Giemsa solution for 90minutes and rinsed in distilled water and dried. Then dried smears were examined at 100X oil immersion objectives. About 200 spermatozoa were counted for acrosome status after staining. This method stains the acrosome dark purple in color.

3.7.2.7 Sperm plasma membrane integrity

Functional membrane integrity of spermatozoa was evaluated by hypo-osmotic swelling test (HOST) as per the method described by Jeyendran *et al.* (1984) and Correa and Zavos, (1994). Hypo-Osmotic solution (150 mOsmol/l) and Control isotonic solution (300 mOsmol/l) was prepared as follows

Table 3.7: Composition of HOST and control solution

Chemicals	HOST solution	Control solution
Sodium citrate (g)	0.735	2.94
Fructose (g)	1.351	5.40
Millipore water up to (mL)	100	100
Osmolarity (mOsm/L)	150	300

Procedure

One mL of hypo-osmotic solution, having an osmotic strength of 150 mOsm/kg was mixed with 0.1 mL of semen and incubated at 37°C for one hour. Following incubation, a drop of well-mixed solution was taken on a clean, dry glass slide and covered with a cover-slip. Sperm tail curling was recorded as an effect of swelling due to the influx of water. A total of about 200 spermatozoa were counted in different fields' at 400X magnification under DIC microscope. The total proportion of swollen spermatozoa was calculated by dividing the number of reacted cells by the total spermatozoa counted in the same area and multiplying the figure by 100. The proportion of swollen spermatozoa from a control sample was subtracted from this value. These spermatozoa were classified into four different classes according to the presence of following swelling pattern (Takahashi *et al.* 1990).

Observations/patterns:

- A. No swelling, no membrane reaction
- B. Swelling of the tip of the tail
- C. Different type of hairpin-like swelling pattern
- D. Complete tail swelling

Spermatozoa displaying B, C or D were considered positive for the HOST test.

3.7.2.8. Total sperm abnormality

Smear prepared for non-eosinophilic sperm count was also used for enumerating total sperm abnormalities. The slides were observed under oil immersion lens. About 200

spermatozoa were counted in different fields and percentage of abnormal spermatozoa was calculated as follows:

$$\text{Total abnormality (\%)} = \frac{\text{Total number of abnormal spermatozoa (Head, mid piece and tail)}}{\text{Total number of spermatozoa counted}} \times 100$$

3.8 Statistical analysis

The data generated from experiments (Meteorological, physiological, hormonal profile, body surface temperature and semen quality) were subjected to statistical analysis by one- way analysis of variance (ANOVA) using SPSS software version 20 and means were compared using Duncan's multiple range test to draw scientific inferences.

CHAPTER -4

Results and Discussion

RESULTS AND DISCUSSION

Artificial insemination technique is being used widely to increase the genetic potential for the production of livestock species including buffalo all over the world. Quality semen from high genetic merit bulls is imperative in improving the fertility and productivity of buffaloes. India has a tropical climate where relative humidity, rainfall and ambient temperature vary significantly within geographical regions. The environmental variables associated with heat stress which affects the physiological responses and thermal regulations are ambient temperature and relative humidity. High environmental temperature is the major constraint for buffalo productivity. Thus the present study was planned for assessment of season and THI induced stress on Murrah buffalo bulls and its effect on testicular temperature, hormonal profile and overall reproductive behavior of bulls.

4.1 Meteorological data of different season

Table 4.1: Meteorological variables(Mean± SE) during different season

Parameter	Winter (15 Dec-15 Feb)	Spring (16 feb-30April)	Summer (1 May-30 June)
Morning temperature (°C)	9.43 ^a ±0.43	17.99 ^b ±0.52	26.6 ^c ±0.29
Afternoon temperature (°C)	19.56 ^a ±0.63	32.19 ^b ±0.58	34.87 ^c ±0.52
Morning relative humidity (%)	98.47 ^a ±0.48	75.75 ^b ±2.75	73.52 ^b ±1.55
Afternoon relative humidity (%)	78.66 ^a ±1.86	41.69 ^b ±2.78	45.82 ^b ±1.87
THI	58.01 ^a ±0.75	72.36 ^b ±0.5	80.79 ^c ±0.38

Means bearing different superscripts in a row differ significantly (p<0.05)

The data regarding climatic conditions like temperature, relative humidity during the study period were taken from Central Soil Salinity Research Institute (CSSRI), Karnal, India. Temperature humidity index (THI) was calculated with the help of aforementioned variables. The means of the recorded data are presented in table 4.1 and fig. 4.1- 4.3

The mean values for ambient temperature (°C) recorded during winter, spring and summer season was 9.43±0.43, 17.99±0.52 and 26.6±0.29 in the morning (6-7AM) and 19.56±0.63, 32.19±0.58 and 34.87±0.52 in the afternoon (2-3PM), respectively. The

Results and Discussion

ambient temperature varied ($p < 0.05$) among different seasons, it was lowest in winter and highest in summer season. The relative humidity (%) during winter, spring and summer season was 98.47 ± 0.48 , 75.75 ± 2.75 and 73.52 ± 1.55 for morning hours and 78.66 ± 1.8 , 41.69 ± 2.78 and 45.82 ± 1.87 in the afternoon. It was higher ($p < 0.05$) in winter season as compared to spring and summer.

The value of THI was highly variable among different seasons it was 58.01 ± 0.75 in winter; 72.36 ± 0.5 in spring and 80.79 ± 0.38 in summer season. It was maximum during summer (80.79 ± 0.38) followed by spring (72.36 ± 0.5) and winter (58.01 ± 0.75) season. THI values indicated that level of stress during different season. Results of this study are in agreement with the Nitharwal (2013), where summer season was the most stressful period for the reproductive performance of Murrah buffalo bulls. Similarly, Sinha (2018) also reported that the THI was maximum during summer and rainy seasons followed by autumn and winter and the results of the study showed that Murrah buffalo bulls felt more stress during rainy and summer season. Sharma *et al.* (2017) reported that the cross bred cattle bulls showed poor semen quality where THI was 78 to 84. Similar to the findings of present study the THI was recorded highest (> 80.88) during summer, medium (70.06-80.88) in rainy season and lowest (< 70.06) in winter season (Ahirwar *et al.*, 2018) in Bangalore region. The semen quality was found to be poor where THI was high in Murrah buffalo bulls.

4.2 Physiological responses in bulls during different season

Table 4.2: Physiological responses (Mean \pm SE) of buffalo bulls during different seasons

Physiological responses RR (breath/min), RT ($^{\circ}$ C)	Winter	Spring	Summer
Morning respiration rate (MRR)	$11.88^a \pm 0.12$	$15.56^b \pm 0.17$	$24.38^c \pm 0.42$
Afternoon respiration rate (ARR)	$14.25^a \pm 0.19$	$19.78^b \pm 0.26$	$29.74^c \pm 0.55$
Morning rectal temperature (MRT)	$36.91^a \pm 0.05$	$37.27^b \pm 0.05$	$37.58^c \pm 0.05$
Afternoon rectal temperature (ART)	$37.19^a \pm 0.40$	$37.83^{ab} \pm 0.03$	$38.16^b \pm 0.06$

Means bearing different superscripts in a row differ significantly ($p < 0.05$)

Physiological parameters such as respiration rate (RR) and Rectal temperatures were recorded in morning and afternoon during winter, spring and summer seasons. The respiration rate (breaths/minute) and rectal temperature (°C) are depicted in table 4.2 and fig 4.4- 4.5

The mean values for rectal temperatures (°C) in morning hours during winter, spring and summer seasons were 36.91 ± 0.05 , 37.27 ± 0.05 and 37.58 ± 0.05 , respectively. Value of rectal temperatures during morning hours was higher ($p < 0.05$) in summer season as compared to spring and winter season. Similarly for afternoon during winter, spring and summer season were 37.19 ± 0.40 , 37.83 ± 0.03 and 38.16 ± 0.06 , respectively. Higher values for rectal temperature in the afternoon were during summer and spring as compared to winter season. The respiration rate (breaths/ minute) was 11.88 ± 0.12 in winter; 15.56 ± 0.17 in spring and 24.38 ± 0.42 in summer during morning hours and 14.25 ± 0.19 in winter, 19.78 ± 0.26 in spring 29.74 ± 0.55 in summer during afternoon hours. An increase ($p < 0.05$) in respiration rate was observed in summer season as compared to spring and winter season

Rectal temperature is thought to be a reliable indicator of body temperature (Srikandakumar and Johnson, 2004). Core body temperature in cattle indicates the temperature of the most important body organs like heart, liver and brain (Farooq *et al.*, 2010). The rectal temperature during summer and winter, was $102.52 \pm 0.25^\circ\text{F}$ and $100.68 \pm 0.19^\circ\text{F}$, respectively and respiration rate was 77 ± 1.25 and 12.29 ± 1.97 breaths/minute, respectively (Singh *et al.*, 2014) in Murrah buffalo and they inferred that an increased respiration during summer compared to winter was an attempt to increase heat loss through evaporative cooling and this may be to meet the more demand of oxygen by the tissues in stressful condition. Dayal *et al.* (2017) studied the effect of season on physiological parameters and found that season had no influence on rectal temperature (winter - $100.3 \pm 0.47^\circ\text{F}$: Summer - $100.2 \pm 0.23^\circ\text{F}$), but it did have an effect ($P < 0.05$) on respiration rate (winter - 13.2 ± 0.4 bpm; summer- 16.9 ± 0.9 bpm). An increase in respiration rate, sweating, rectal temperature and blood flow to skin due to vasodilatation of blood capillaries was found in summer (Whittow, 1965). Moran (1973) also reported that there was an increase in rectal temperature and skin temperature under solar radiation in buffaloes, but it decreased rapidly in contrast when animals were moved into the shade (Badreldin and Ghany, 1954).

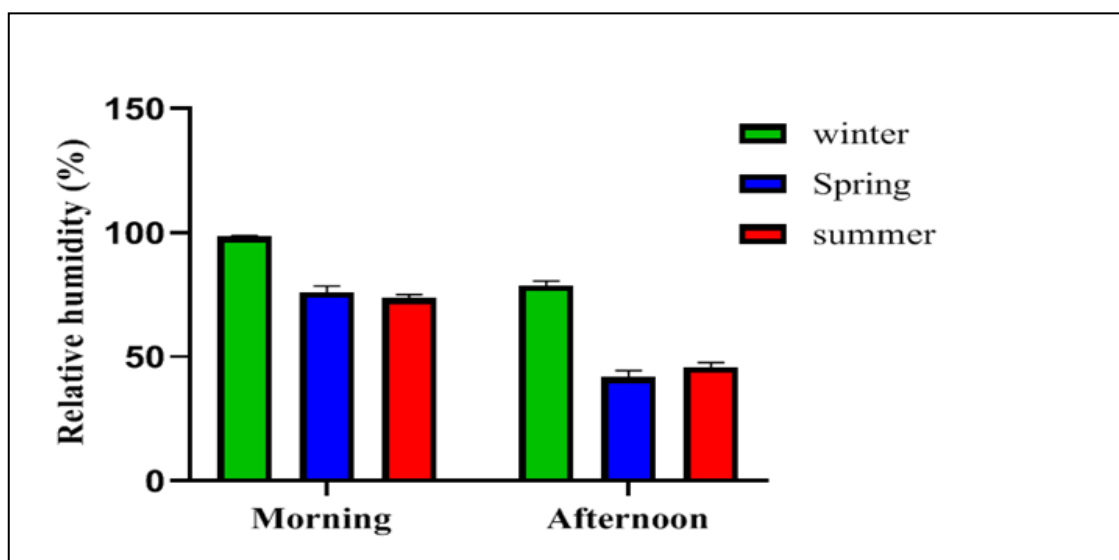


Fig. 4.1: Relative humidity during different season

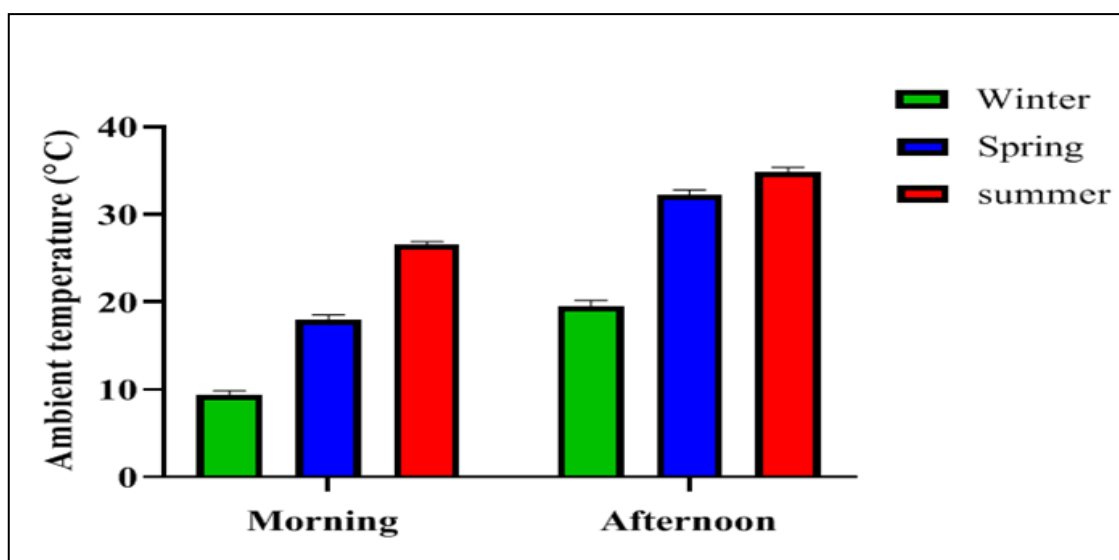


Fig. 4.2: Ambient temperature in morning and afternoon during different seasons

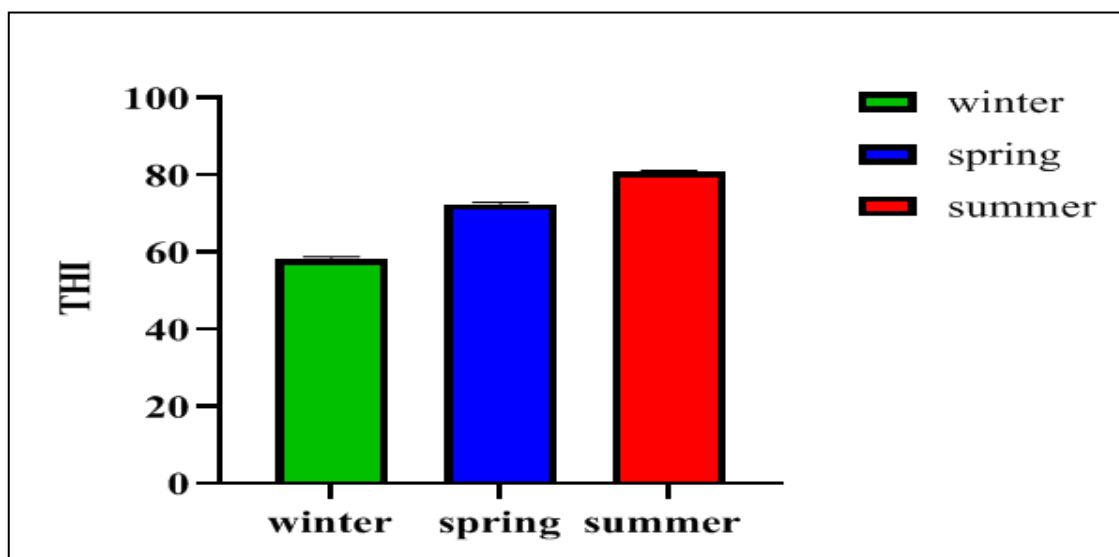


Fig. 4.3: THI during different seasons

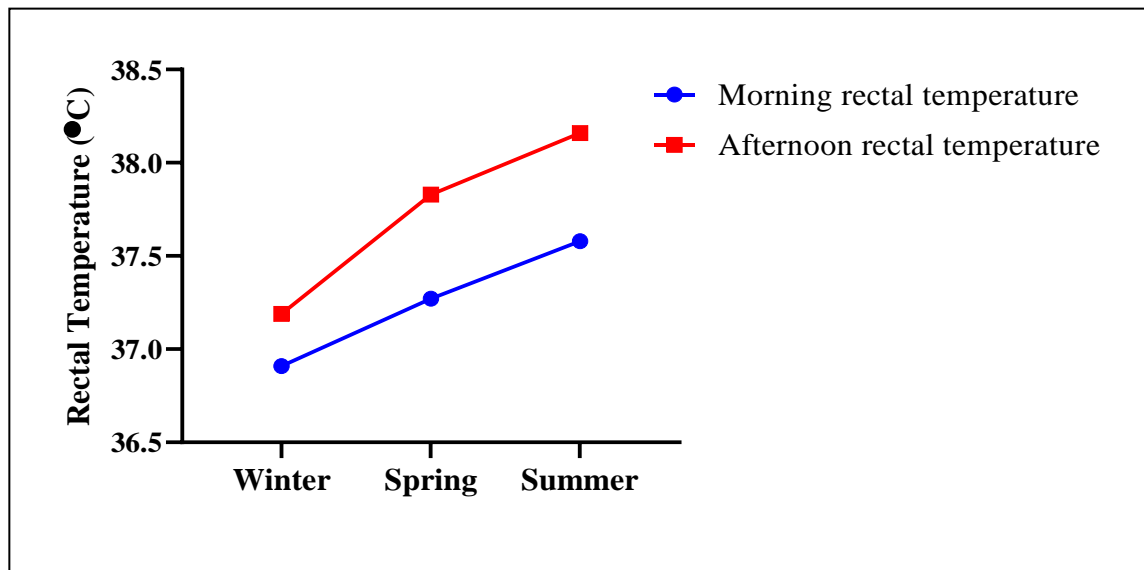


Fig. 4.4 Morning and afternoon rectal temperature of buffalo bulls during different seasons

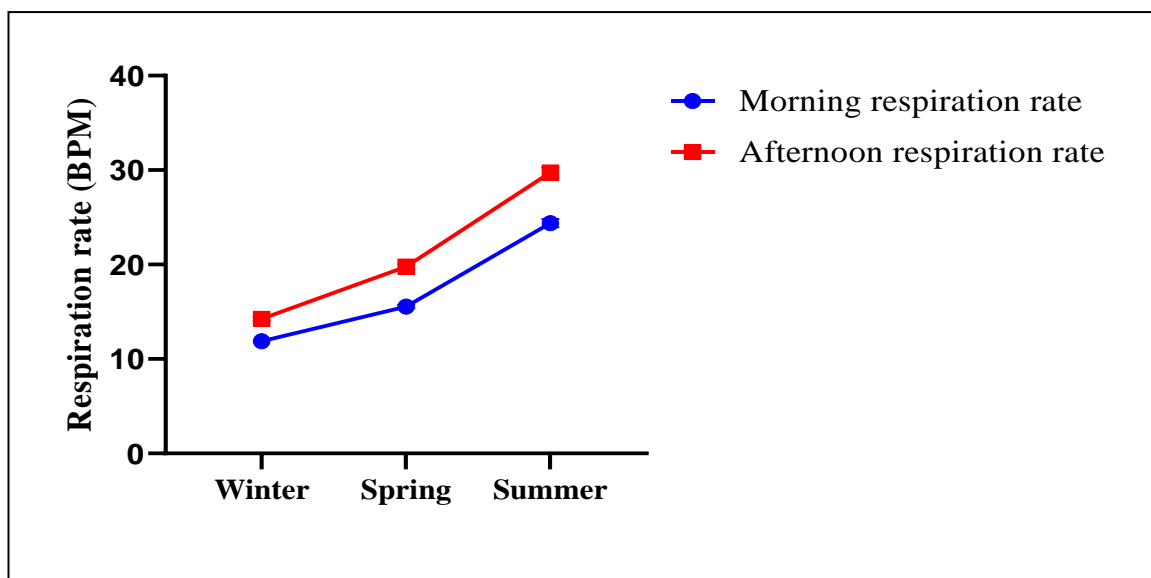


Fig. 4.5: Respiration rate of buffalo bulls during different season

The increase in respiration rate during summer is an indicator of heat stress and provides an easy means of non invasive and distant assessment of heat stress (Silanikove, 2000). Animals modify their behavioral, physiological and metabolic responses to cope up with change in the environmental conditions and to maintain body homeostasis (Rashamol *et al.*, 2020). So from the above observations it can be said that the increase in respiration rate was a compensatory mechanism for heat loss by evaporative cooling in summer heat stress.

4.3 Body surface temperature (°C) of buffalo bulls during different season

Surface temperature of different body parts was recorded to assess the thermal control or stress during different seasons of the year; the values are depicted in table 4.3 and fig. no. 4.6-4.9. The surface temperature (°C) recorded in the morning hours was 33.51±0.52, 34.2±0.21 and 36.77±0.14 for eye; 25.4±0.58, 27.58±0.57 and 34.4±0.36 for forehead; 26.98±0.42, 29.47±0.5 and 34.79±0.26 for flank region and 26.86±0.28, 28.43±0.54 and 34.9±0.28 for rump region during winter, spring and summer season respectively. Similarly temperature recorded during afternoon was 36.17±0.24, 36.17±0.24 and 36.17±0.24 for eye; 32.6±0.36, 35.42±0.36 and 39.1±0.23 for forehead; 34.14±0.4, 35.44±0.43 and 38.56±0.25 for flank and 35±1.34, 36.33±0.35 and 39±0.23 for rump region during winter, spring and summer season, respectively.

Recorded data for all the body surfaces showed significant ($p < 0.05$) difference during different seasons, which was lowest in winter and highest in summer season. Das *et al.*, (1997) also reported that the skin temperature increased as the intensity of solar radiation was increased. Similar to our findings body temperature (BT) was influenced ($P < 0.05$) by season, with the highest value recorded in summer (35.26 °C), moderate in spring (33.93 °C) and the lower in winter (33.44 °C) in Friesian young bulls (Sayah *et al.*, 2019). In hot environmental conditions the body temperature of animals increases (Vermunt and Tranter, (2011) and to cope up with these conditions animals attempt dissipating heat through different physiological means like through increased peripheral circulation, vasodilatation, and sweating, that are coordinated by hypothalamus (Veissier *et al.*, 2018). Temperature of eye (medial canthus) recorded by IRT reflects the core body temperature in cattle (Gloster *et al.*, 2011). It might be due to its location close to the hypothalamus (thermosensitive site) which reduces lag time in response. Stewart *et al.* (2007) and McGreevy *et al.* (2012) reported that eyes (*Caruncula lacrimalis*) and posterior border of the eyelids have superficial capillaries which are highly innervated by the sympathetic nerve fibers.

Table 4.3: Mean± SE of body surface temperature (°C) in buffalo bulls during different seasons

Season	Eye temperature		Forehead temperature		Flank temperature		Rump temperature	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Winter	33.51 ^a ±0.52	36.17 ^a ±0.24	25.4 ^a ±0.58	32.6 ^a ±0.36	26.98 ^a ±0.42	34.14 ^a ±0.4	26.86 ^a ±0.28	35 ^a ±1.34
Spring	34.2 ^a ±0.21	36.84 ^b ±0.14	27.58 ^b ±0.57	35.42 ^b ±0.36	29.47 ^b ±0.5	35.44 ^b ±0.43	28.43 ^b ±0.54	36.33 ^a ±0.35
Summer	36.77 ^b ±0.14	38.15 ^c ±0.14	34.4 ^c ±0.36	39.1 ^c ±0.23	34.79 ^c ±0.26	38.56 ^c ±0.25	34.9 ^c ±0.28	39 ^b ±0.23

Means bearing different superscripts in a column differ significantly (p<0.05)

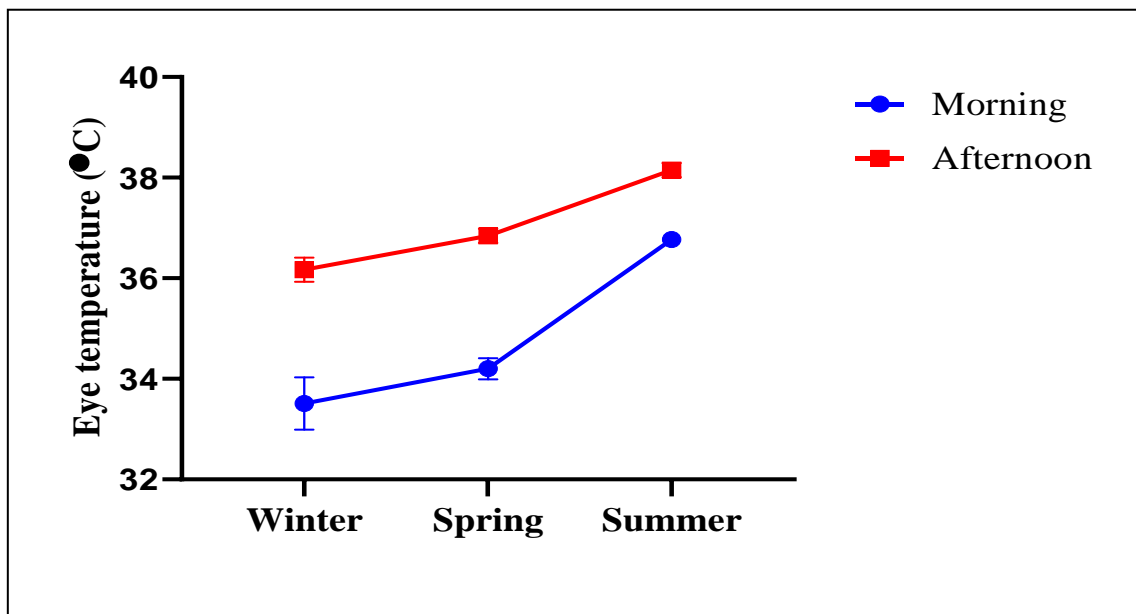


Fig. 4.6: Eye temperature of buffalo bulls during different seasons

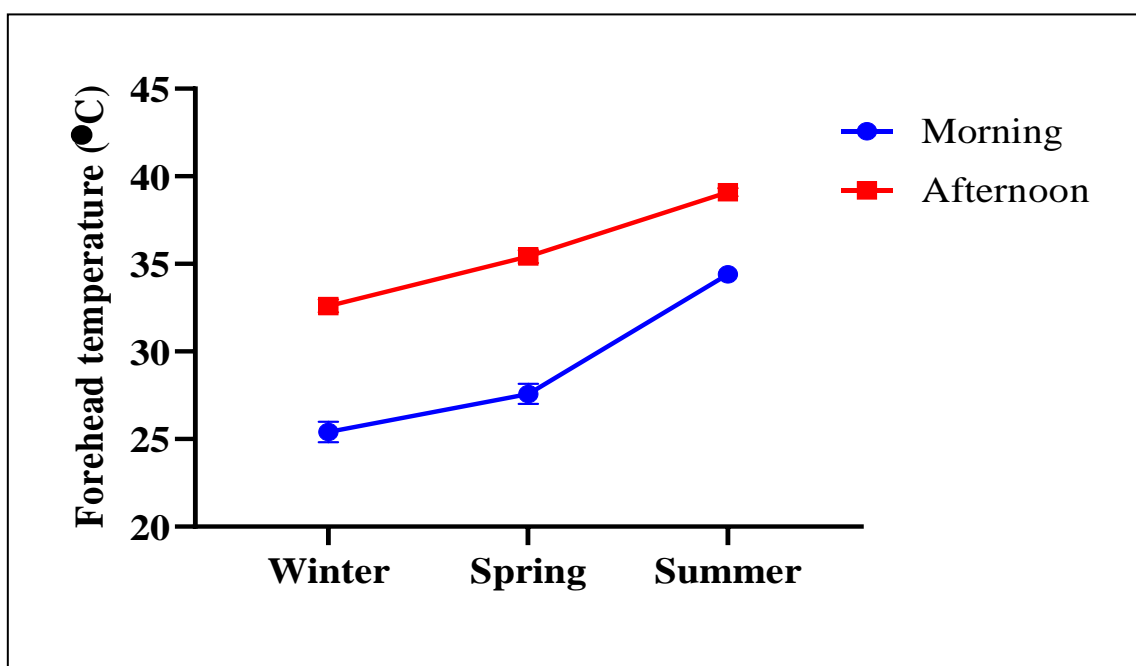


Fig. 4.7: Forehead temperature of buffalo bulls during different seasons

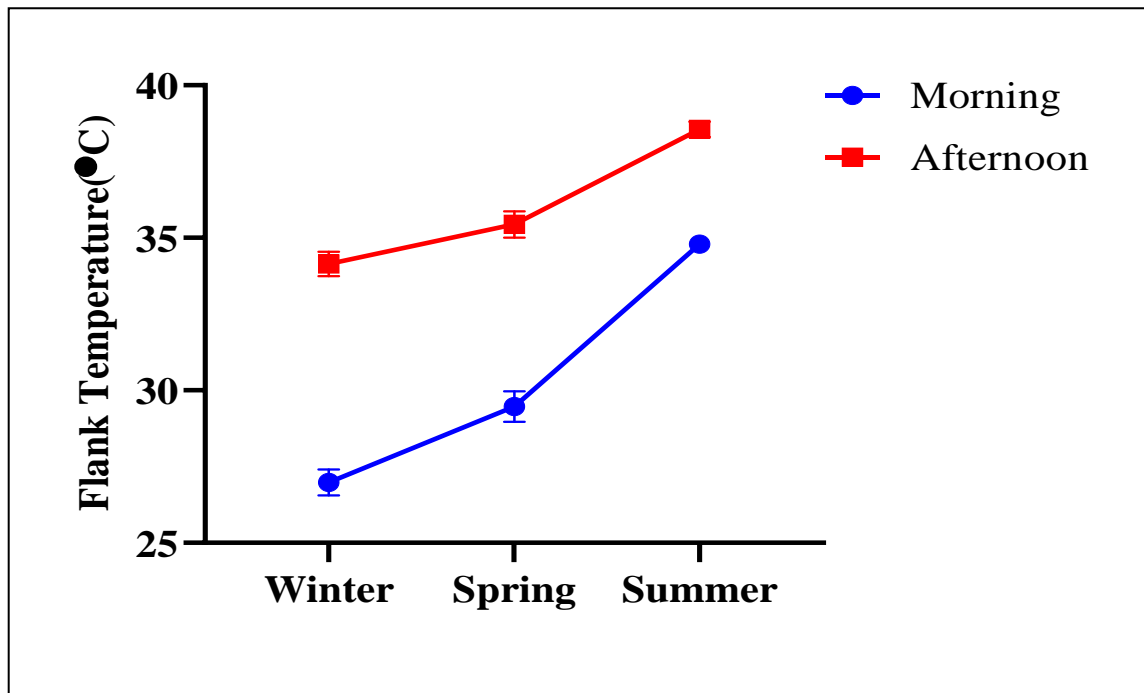


Fig. 4.8: Flank temperature of buffalo bulls during different seasons

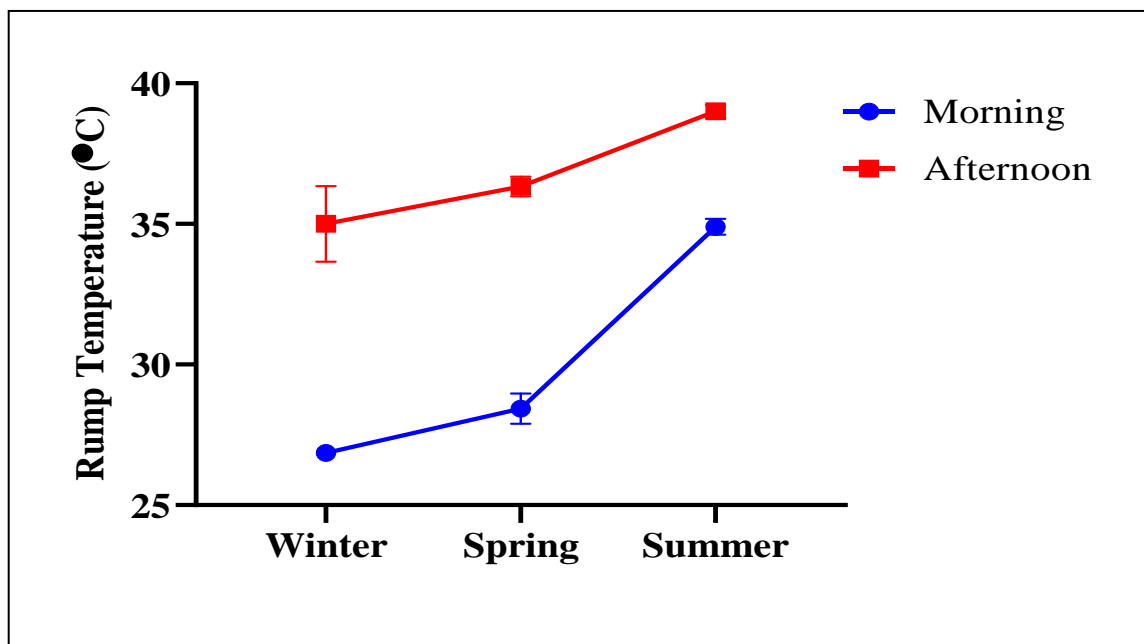


Fig. 4.9: Rump temperature of buffalo bulls during different seasons

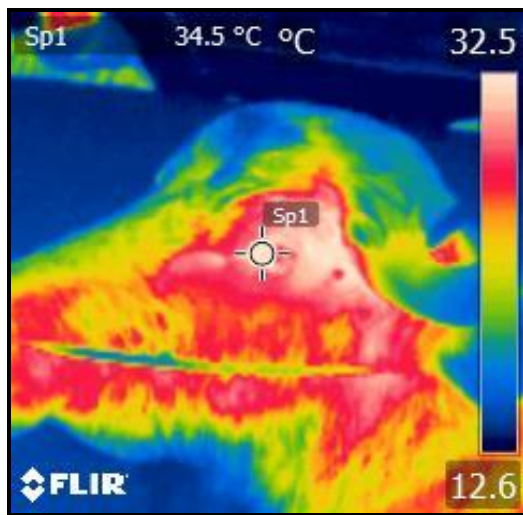


Plate no. 1
Infrared image of eye

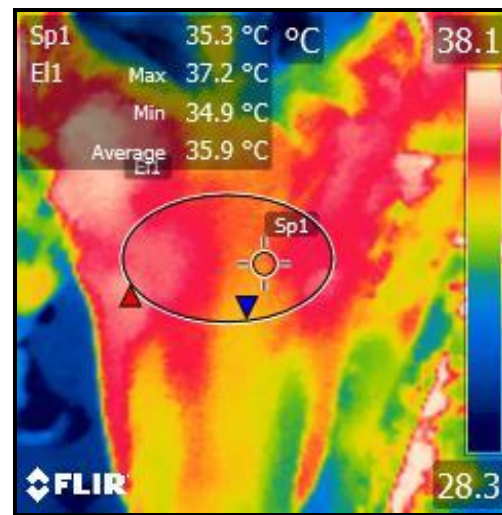


Plate no. 2
Infrared image of forehead

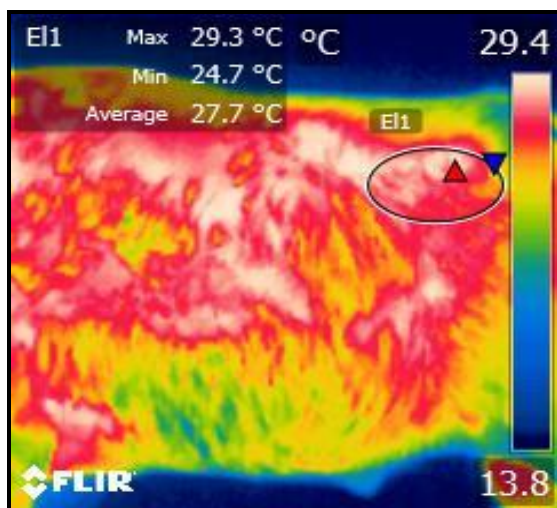


Plate no. 3
Infrared image of flank region

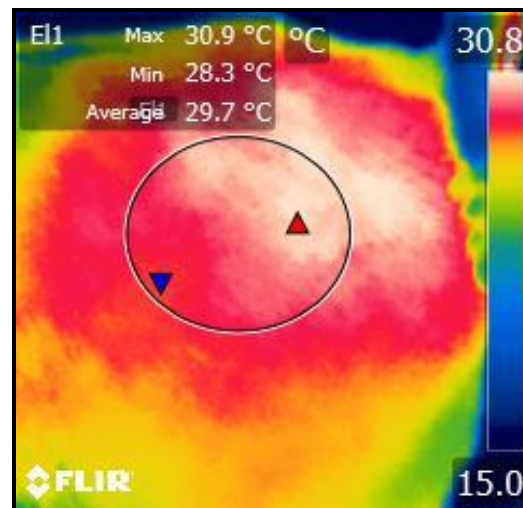


Plate no. 4
Infrared image of rump region

Moreover, eyes have retinal blood vessels similar to the important brain vessels, choroid vessels and ciliary processes, which are similar to the small intestine and kidney. Similar to present study Sinha (2018) also reported significantly higher body surface temperature during summer as compared to winter. The Infrared thermographic technique can be employed to measure the emissions of radiated heat from the external body surfaces. The temperature of external body surface is better associated with changes in the environmental temperature, behavioral responses of emotions, lactation variables, and metabolism (Uddin *et al.*, 2019, 2020). The IRT showed that the temperature of every external body surface varies according to conduction, blood flow, local tissue metabolism and an ability of an object to absorb and emit radiation (Taylor *et al.*, 2014). IRT can be used as a non-invasive tool to record body surface temperature to assess the thermal stress due to environmental factors.

4.4 Scrotal surface temperature (°C) of buffalo bulls during different seasons

The scrotal surface temperature recorded at different points is depicted in table 4.4 and fig. no. 4.11 and 4.12. The scrotal surface temperature (°C) at upper, mid and lower point/part during morning hours was 32.79 ± 0.24 , 30.79 ± 0.30 and 27.18 ± 0.18 , respectively in winter; 33.51 ± 0.17 , 31.61 ± 0.30 and 26.88 ± 0.23 in spring and 35.86 ± 0.20 , 34.0 ± 0.21 and 30.49 ± 0.32 , respectively during summer season. During afternoon hours it was 34.25 ± 0.27 , 32.68 ± 0.32 and 29.91 ± 0.36 during winter; 35.6 ± 0.19 , 34.1 ± 0.19 and 31.05 ± 0.35 , respectively during spring and 37.7 ± 0.17 , 36.2 ± 0.20 and 33.55 ± 0.25 , respectively during summer season. The scrotal temperature varied ($p < 0.05$) among the different location across the season, lowest temperature was in winter followed by spring and summer season.

The temperature gradient (TG) is the difference between the temperature of upper and lower scrotal surface. Testicular temperature gradient is depicted in table no. 4.4 and fig. no. 4.10. The temperature gradient (°C) in the morning during winter, spring and summer was 5.61 ± 0.16 , 6.63 ± 0.25 and 37 ± 0.23 , respectively and 4.34 ± 0.33 , 4.54 ± 0.28 and 4.15 ± 0.19 , respectively during afternoon hours. The temperature gradient was highest during morning hours in spring season whereas it did not differ in afternoon hours across the seasons.

Table 4.4: Mean± SE of scrotal surface temperature (°C) in buffalo bulls during different seasons

Season	Upper surface		Mid surface		Lower surface		Scrotal surface temperature gradient (°C)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Winter	32.79 ^a ±0.24	34.25 ^a ±0.27	30.79 ^a ±0.30	32.68 ^a ±0.32	27.18 ^a ±0.18	29.91 ^a ±0.36	5.61 ^a ±0.16	4.34±0.33
Spring	33.51 ^b ±0.17	35.6 ^b ±0.19	31.61 ^b ±0.30	34.1 ^b ±0.19	26.88 ^a ±0.23	31.05 ^b ±0.35	6.63 ^b ±0.2	4.54±0.28
Summer	35.86 ^c ±0.20	37.7 ^c ±0.17	34.0 ^c ±0.21	36.2 ^c ±0.20	30.49 ^b ±0.32	33.55 ^c ±0.25	5.37 ^a ±0.23	4.15±0.19

Means bearing different superscripts in a column differ significantly (p<0.05)

The Infrared thermography is a simple, non-contact and non-invasive technique to take scrotal surface temperature in bulls (Kastelic *et al.*, 1997; Menegassi *et al.*, 2015). Thus, this technology can be used as a general indicator of stress. In scrotum having apparently normal testicular thermoregulation, temperature decreases from dorsal to ventral area and presents the scrotal surface temperature gradients between 4 and 6 °C (Kastelic, 2014). The present findings for scrotal surface temperature are in accordance with Sinha (2018) it was reported that the temperature of proximal pole was higher in all the season than the distal pole and the temperature gradient was maximum during winter ($3.66\pm 0.10^{\circ}\text{C}$) and minimum during summer season ($2.44\pm 0.08^{\circ}\text{C}$). Yadav *et al.* (2017) reported that the bulls with a higher temperature gradient produced better quality sperm than those with a lower temperature gradient. Similarly Kushwaha *et al.* (2018) found that as scrotal surface temperature gradient (SSTG) increased mass activity and non-eosinophilic sperm count were also dramatically enhanced, while sperm abnormalities were decreased ($p < 0.01$). Menegassi *et al.* (2015) used infrared thermography to assess the thermoregulation and spermatogenic alterations due to scrotal temperature gradient in testicular compromised bulls (9 Brangus breed bulls 5/8 Angus * 3/8 Nelore). Scrotum was insulated for 72 hours ($n = 6$) and control animals ($n = 3$) were not insulated during the trial. It was found that two weeks after treatment sperm motility was poor in scrotal insulated animals compared to control animals and total spermatozoa defects were higher in insulated animals ($p < .05$). Similarly, Ahirwar *et al.* (2018) used scrotal infrared digital thermography to evaluate effects of thermal stress on semen quality of Murrah buffalo (*Bubalus bubalis*) breeding bulls, it was reported that high THI leads to decrease in testicular gradient and ultimately led to poor quality of semen. From the above observations it can be concluded that elevated scrotal and testicular temperature (decreased testicular gradient) leads to poor quality of semen.

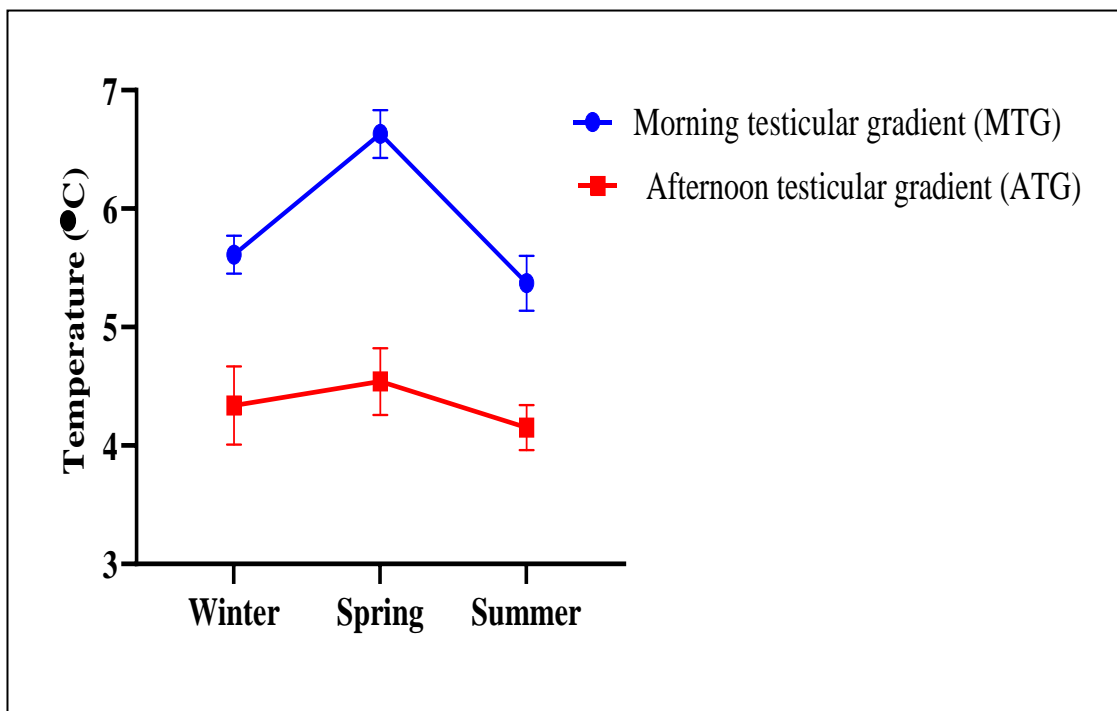


Fig. 4.10: Testicular temperature gradient of buffalo bulls during different seasons

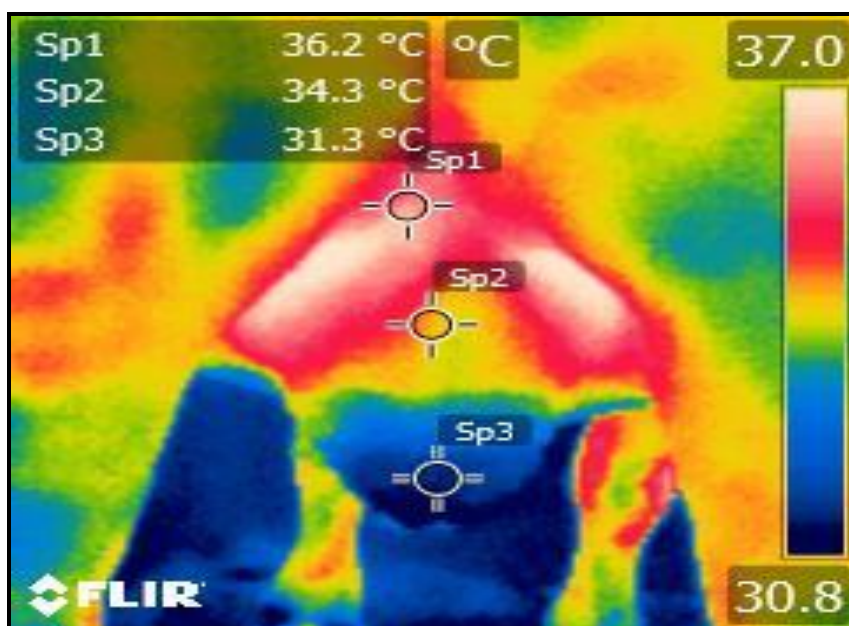


Plate no. 5
Infrared image of scrotum

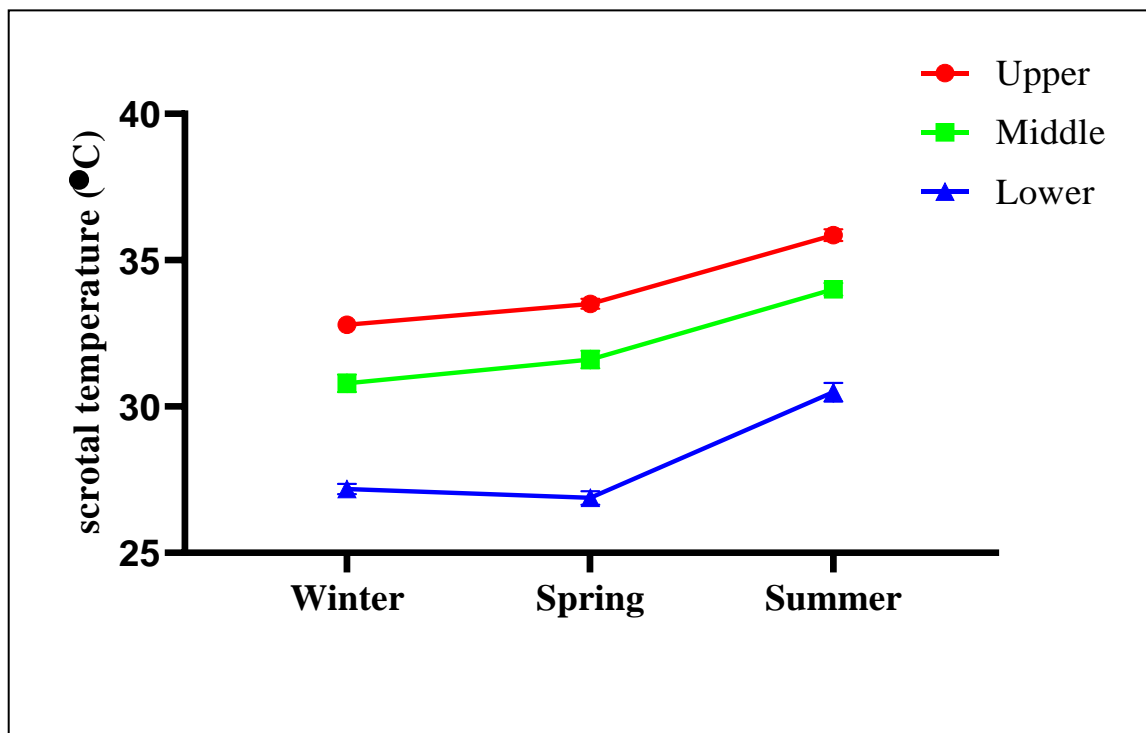


Fig. 4.11: Scrotal surface temperature (morning) of buffalo bulls during different seasons

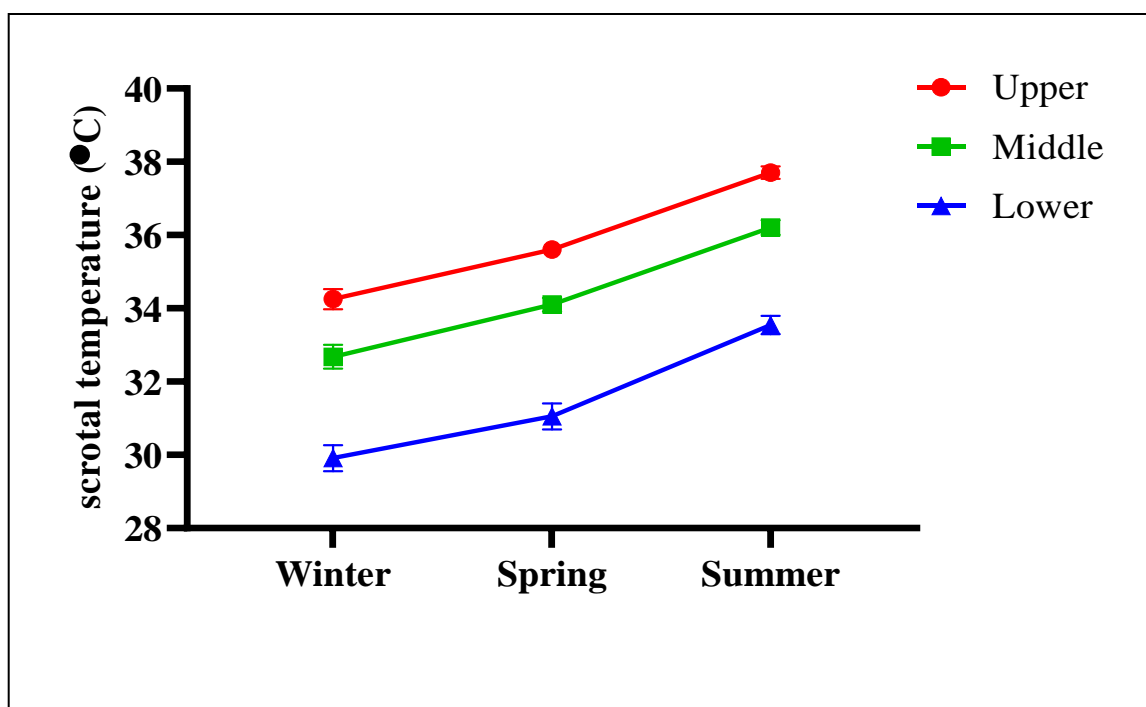


Fig. 4.12: Scrotal surface temperature (afternoon) of buffalo bulls during different seasons

4.5 Endocrinological profile of buffalo bulls during different seasons

The values of endocrinological profile of Murrah buffalo bulls during different season are depicted in table 4.5 and fig. 4.13 - 4.17

The mean TSH ($\mu\text{IU/mL}$) concentration during different seasons was higher ($P<0.05$) in winter (0.020) and spring (0.016) season as compared to summer and the concentration of T3 (ng/mL) was 4.98 ± 0.21 in winter, 4.28 ± 0.14 in spring and 1.79 ± 0.31 during summer season. Higher ($P<0.05$) value of T3 was in winter season and least during summer season. Similarly the mean concentration of T4 (ng/mL) hormone was 54.54 ± 1.77 in winter; 53.24 ± 1.88 in spring and 37.71 ± 1.05 in summer with lower ($P<0.05$) value in summer season and higher during winter and spring season was obtained. Results of a study conducted by Mayahi *et al.* (2014) on bulls are in accordance with present values for blood serum T3 uptake which was higher in winter than summer ($p\leq 0.05$) season but the differences of T3 and TSH concentrations did not show any significant difference among seasons. Along with this the seasonal variation in levels of thyroid hormone and their relation with semen quality in Murrah buffalo bulls was studied by Dixit *et al.* (1984), where level of T3 and T4 did not showed any significant differences between seasons. In another investigation by Perumal *et al.* (2017) on Mithun bulls level of thyroxin was considerably higher ($P<0.05$) in the spring and winter seasons than in the summer season. Dayal *et al.* (2017) also studied seasonal variation in T3 and T4 level in Murrah buffalo but this variation was statistically non-significant. Higher Level of T3 and T4 was recorded in winter than the summer. Thyroid hormones are known to play role in thermogenesis in animals and their adaptability to changing environment. Decreased level of thyroid hormones during summer in the present study may be due to depression in activity of thyroid gland due to heat stress.

The mean of cortisol (ng/ml) concentration in the present study was 4.59 ± 1.21 during winter; 3.41 ± 0.62 in spring and 4.24 ± 0.42 in summer season. The concentration of cortisol was higher during winter months. Similar to the present findings Perumal *et al.* (2017) also reported lower ($P<0.05$) level of cortisol in the spring and winter seasons than in the summer season. No significant difference in cortisol concentration was reported by Mallick *et al.* (2016) in Karan fries bulls. Dayal *et al.* (2017) found significant effect ($P<0.05$) of season on cortisol level (summer; 5.34 ± 0.24 ng/ml Vs. winter; 3.76 ± 0.21 ng/ml) in Murrah buffalo and it was concluded that this may be due to activation of the hypothalmo-pituitary-adrenocortical Axis (HPA) and sympatho-adrenal

medullary axis in the heat stress condition. In the present study the non-significant change in the level of cortisol hormone may be due to well adaptability of animals to the surrounding environmental condition during the period of experiment. The bulls were given standard management by providing one time showering and they were kept under proper shaded area.

The testosterone (ng/mL) concentration was significantly higher ($P < 0.05$) during summer (2.41 ± 0.37) as compared to spring (1.46 ± 0.18) and winter (1.09 ± 0.16). The similar seasonal variation was obtained by Barnabe *et al.* (1995), they reported the testosterone concentration ranging from 0.10-1.36 ng/ml in winter and from 0.10-2.54 ng/ml in summer during the 24-hour sampling in Jaffarabadi x Mediterranean buffalo bulls. Perera *et al.* (1979) also reported the seasonal differences in the plasma concentration of testosterone hormone in Murrah buffalo bulls over a period of 10 h (06.00 to 16.00 h) and the mean testosterone concentration reported was highest during August, falling through December to April to reach the lowest value in May. Contrary to this Minton *et al.* (1981) estimated the level of testosterone hormone in Angus bulls exposed to elevated temperature, results of study showed that there was slight decrease in testosterone concentration in the bulls exposed to elevated temperature after 15 days of exposure. Similar to the present findings Dixit *et al.*, (1984) reported that the Murrah buffaloes bulls showed significantly lower values for testosterone hormone during winter (0.53 ± 0.06 ng/ml) than during summer (1.22 ± 0.19 ng/ml) season. Similarly Mallick *et al.* (2016) also reported higher level of testosterone during summer (7.01 ± 0.92 ng/ml) as compared to winter (3.80 ± 0.31 ng/ml) season in cross-bred (Karan fries) bulls and they concluded that increased peripheral testosterone concentration during summer may be due to the stimulatory effect of photoperiod in summer season. No seasonal difference in blood serum testosterone level was reported by Mahmood *et al.* (2013) in Cholistani AI bulls, they found that the decreased concentration of testosterone during winter may be due to depressed hypothalamic-hypophyseal axis depressed due to cold stress. Godfrey *et al.* (1990) reported significant variation in serum testosterone concentration, being lower in winter in Brahman bulls. In contrary Chacur *et al.* (2013) reported a decrease in testosterone levels in summer in Nellore bulls as compared to winter possibly associated with a higher average temperature (26.8°C) in summer and in winter season associated with decreased quality of grasses. However, Perry *et al.* (1991) reported the non-significant change in the level of testosterone hormone during different season and concluded that the reason for harmonious endocrine pattern may be due to

Results and Discussion

well adapted state of animal in existing environmental condition. Stumpf *et al.* (1993) studied the influences of season of a year on concentration of testosterone in circulation of the bovine male; they found that the mean concentration of testosterone was highest at the summer solstice and lowest at the winter solstice. Peirce *et al.* (1987) reported elevated Serum prolactin (PRL) concentrations in Holstein, Brahman and Hereford bulls during the summer months this change in level of prolactin is thought to be due to effect of photoperiodism and temperature (Tucker, 1974). In bulls elevated level of prolactin corresponds with the high level of testosterone. Bartke (1986) reported the stimulatory effect of prolactin in testicular function. This increased PRL increases the sensitivity of Leydig cells in testis for LH through PRL-induced increases in concentration of LH receptors in the testis (Klemcke *et al.*, 1984) this increase in receptivity for LH in testis during summer season is thought to be a cause of increase in level of testosterone in bulls during summer season.

Table 4.5: Endocrinological profile (Mean± SE) of buffalo bulls during different seasons

Season	T3 (ng/mL)	T4 (ng/mL)	TSH (μ IU/mL)	Cortisol (ng/mL)	Testosterone (ng/mL)
winter	4.98 ^a ±0.21	54.54 ^a ±1.77	0.020 ^a ±00	4.59±1.21	1.09 ^a ±0.16
spring	4.28 ^a ±0.14	53.24 ^a ±1.88	0.020 ^a ±00	3.41±0.62	1.46 ^a ±0.18
summer	1.79 ^b ±0.31	37.71 ^b ±1.05	0.016 ^b ±00	4.24±0.42	2.41 ^b ±0.37

Means bearing different superscripts in a column differ significantly ($p < 0.05$)

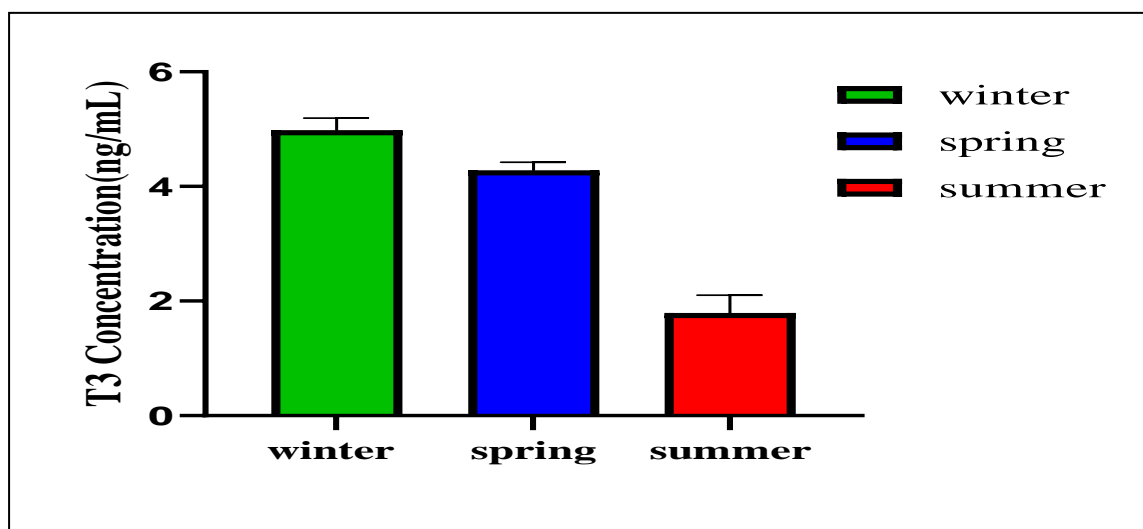


Fig. 4.13: Concentration of T3 hormones of buffalo bulls during different seasons

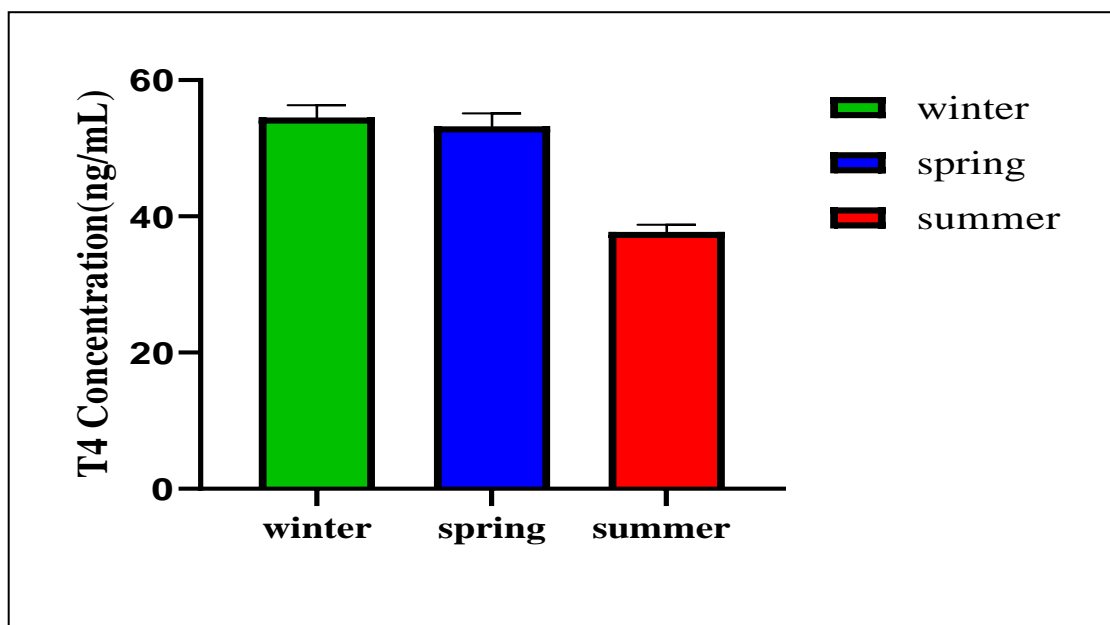


Fig. 4.14: Concentration of T4 hormones of buffalo bulls during different seasons

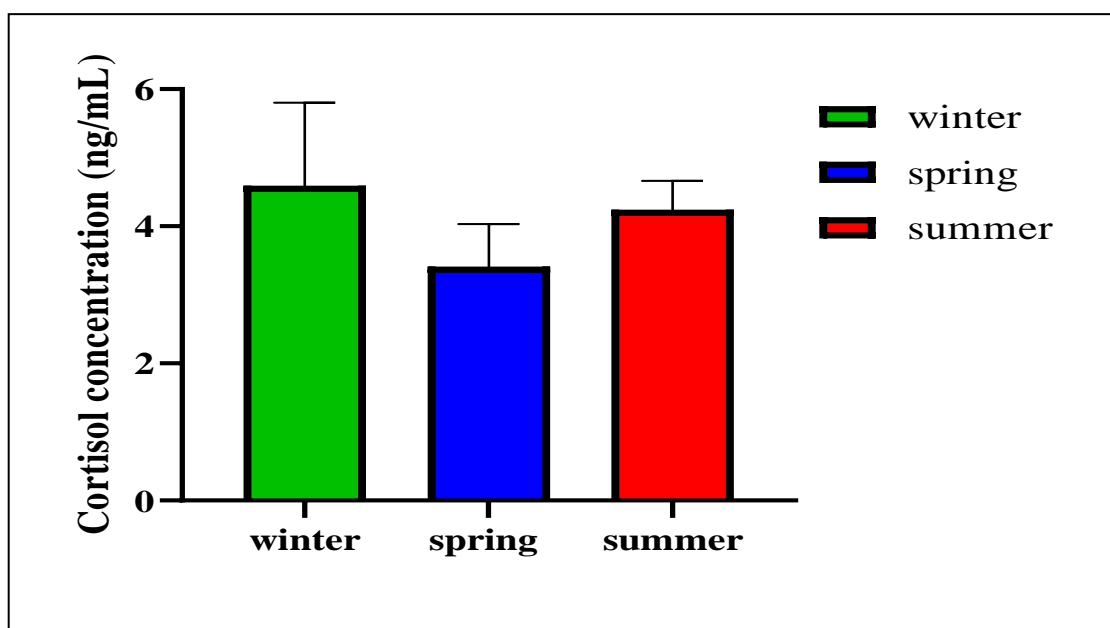


Fig. 4.15: Level of cortisol hormone of buffalo bulls during different seasons

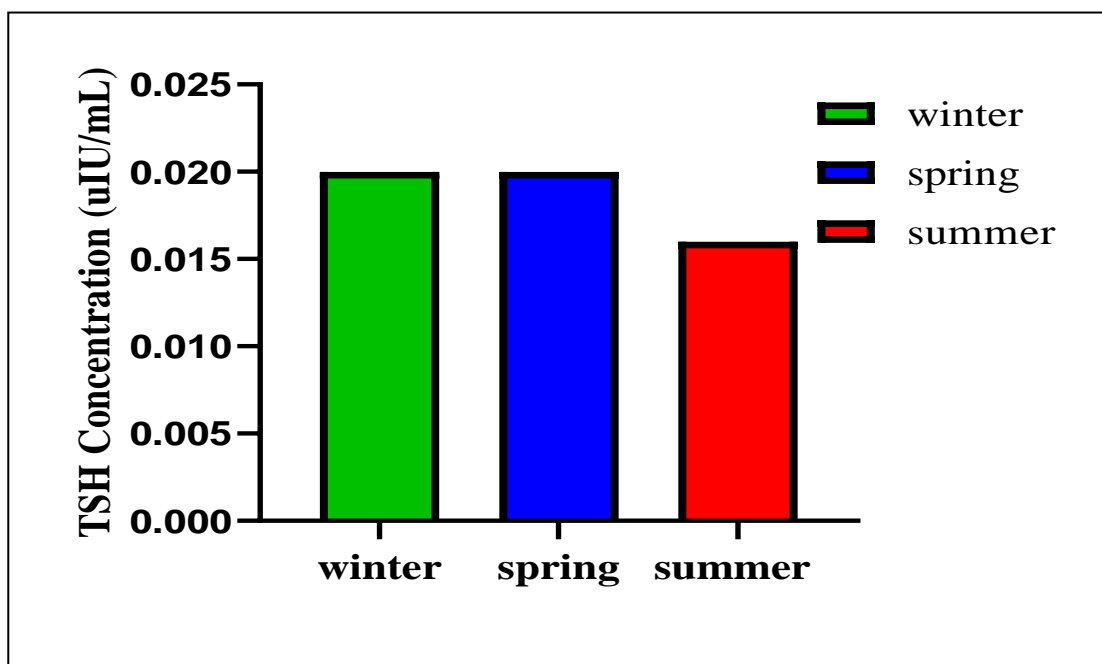


Fig. 4.16: TSH concentration of buffalo bulls during different seasons

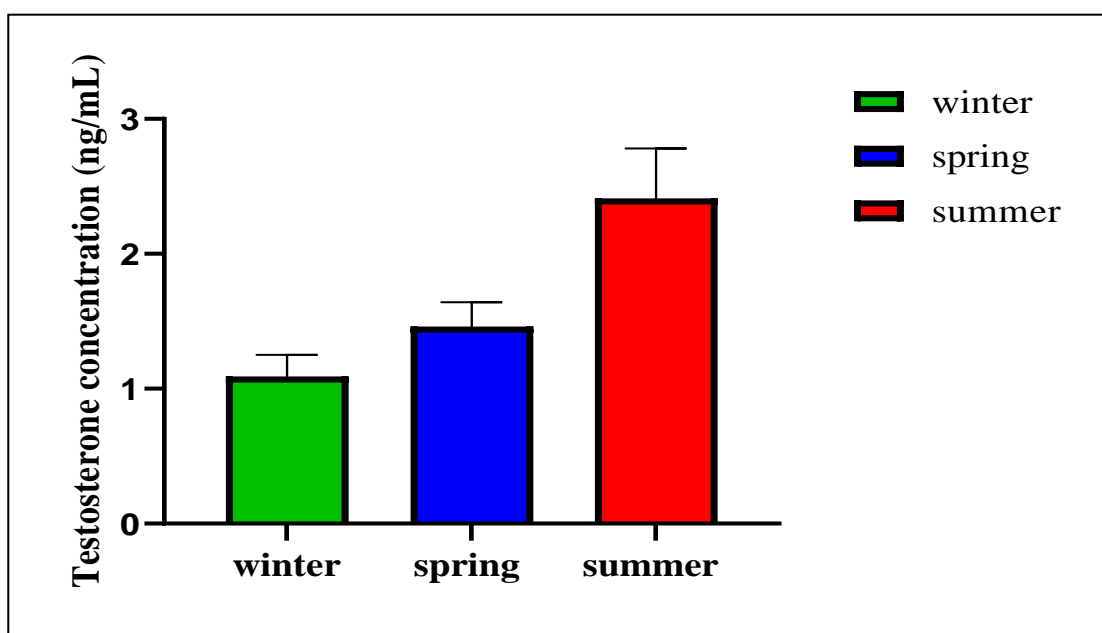


Fig. 4.17: Level of testosterone hormone of buffalo bulls during different seasons

4.6 Sexual behavior of buffalo bulls during different season

The results of seasonal variation in sexual behavior of buffalo bulls are presented in table 4.6 and 4.18- 4.20. The mean of reaction time of bulls was 264.57 ± 13.91 in summer; 225.07 ± 12.96 in spring; 248.57 ± 12.85 in winter. The reaction time did not vary among the season whereas longer reaction time was observed in summer than winter and least during spring season. In similar trend longer reaction time (min) was recorded during summer season (2.78 ± 0.41) than spring (2.46 ± 0.43), autumn (2.54 ± 0.65) and winter (2.75 ± 0.52) seasons in Sahiwal bulls by Ahmad *et al.* (2005). Mahmood *et al.*, (2013) also reported no differences in mean reaction time (min) during autumn (4.59 ± 0.66); winter (3.82 ± 0.51); dry summer (5.11 ± 0.66); and wet summer (4.89 ± 0.60) in 6 Cholistani AI bulls, they concluded that difference in breed could be attributed to these variations. Ramadan *et al.* (2009) reported highest Reaction time (sec.) during summer followed by autumn, spring and winter. Misra and Sengupta (1965) also reported that heat stress lead to rapid loss of libido in buffalo bulls during summer season. Results of present study indicate that bulls' libido decreased with increase in THI and this is in accordance with Ramadan *et al.* (2009) who reported significant ($P < 0.01$) positive correlations between reaction time and both ambient temperature and THI.

The mean values for libido score (LS) of Murrah buffalo bulls was significantly higher ($P < 0.05$) during the spring (38 ± 1.72) and winter (36.71 ± 1.48) season as compared to summer (29.17 ± 3.04). Significantly ($P < 0.05$) lower libido score was reported by Ahmad *et al.* (2005) during summer season (2.95 ± 0.19) than during autumn (3.32 ± 0.19), winter (3.37 ± 0.21) and spring (3.39 ± 0.21) seasons in Sahiwal bulls. In present study Bulls with better libido score in spring produced more number of viable sperm than summer season.

The Murrah buffalo bulls included in the study showed significantly ($p < 0.05$) higher values for total time taken to ejaculate during summer (273.36 ± 13.15) as compared to spring (231.21 ± 13) and winter (254.21 ± 12.67). This increase in total time taken to ejaculate in bulls during summer season may be due to decrease in libido and increased reaction time in summer due to heat stress.

Table 4.6: Effect of season on sexual behaviour (Mean± SE) of Murrah buffalo bulls

Sexual behavior attributes	Summer	Spring	Winter
Reaction time (RT)	264.57±13.91	225.07±12.96	248.57±12.85
Libido score (LS)	29.17 ^a ±3.04	38 ^b ±1.72	36.71 ^b ±1.48
Total Time taken to ejaculate	273.36 ^a ±13.15	231.21 ^b ±13.19	254.21 ^{ab} ±12.67

Means bearing different superscripts in a row differ significantly (p<0.05)

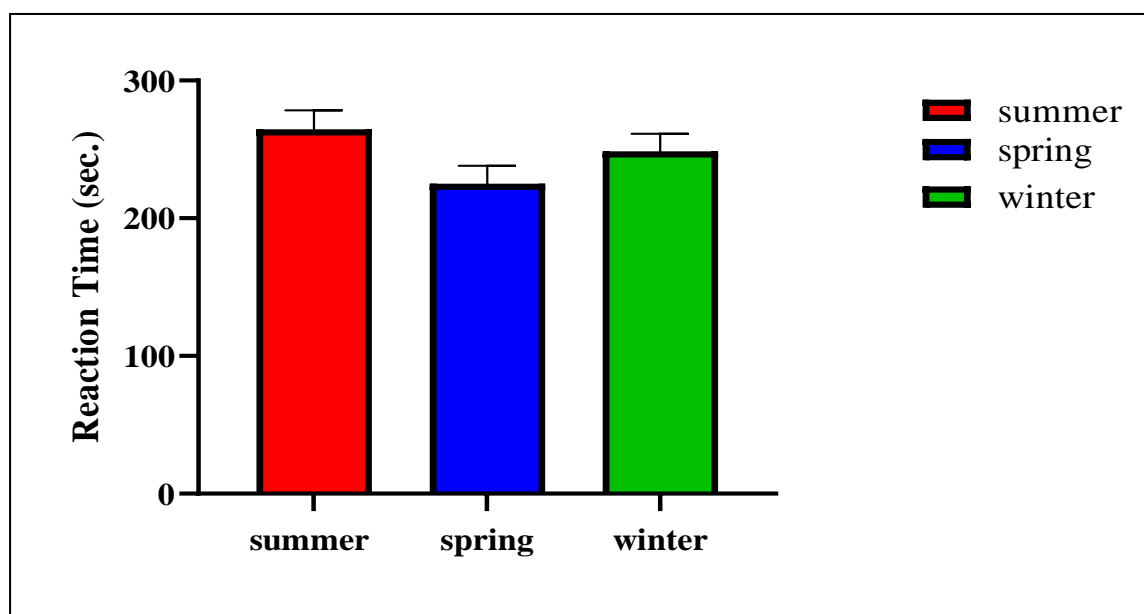


Fig. 4.18: Reaction time in buffalo bulls during different seasons

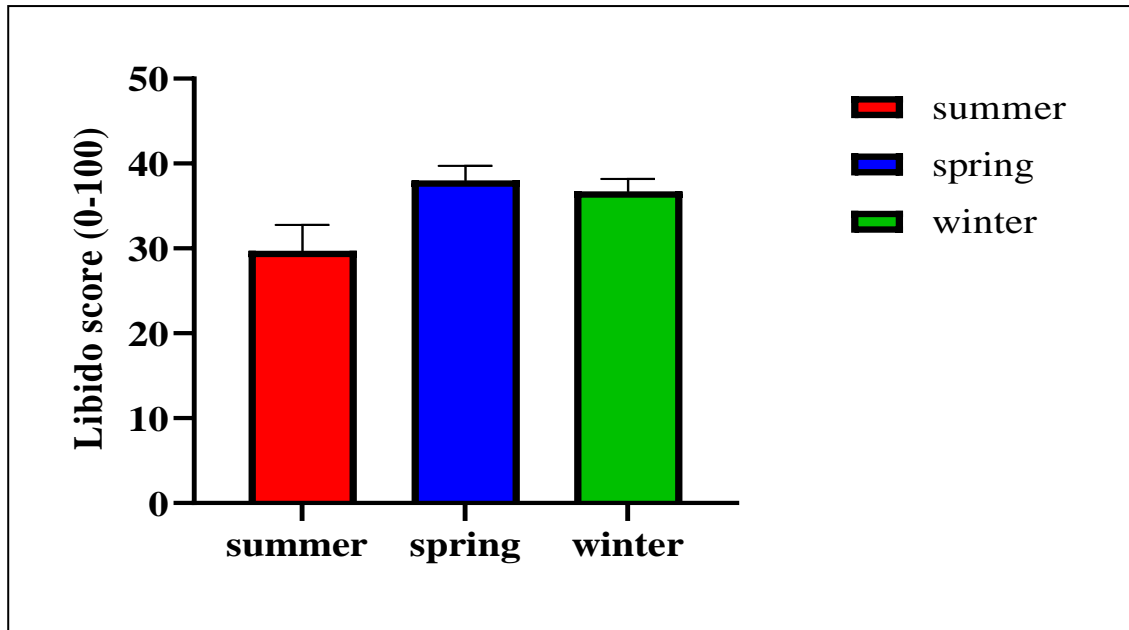


Fig. 4.19: Libido score of buffalo bulls during different seasons

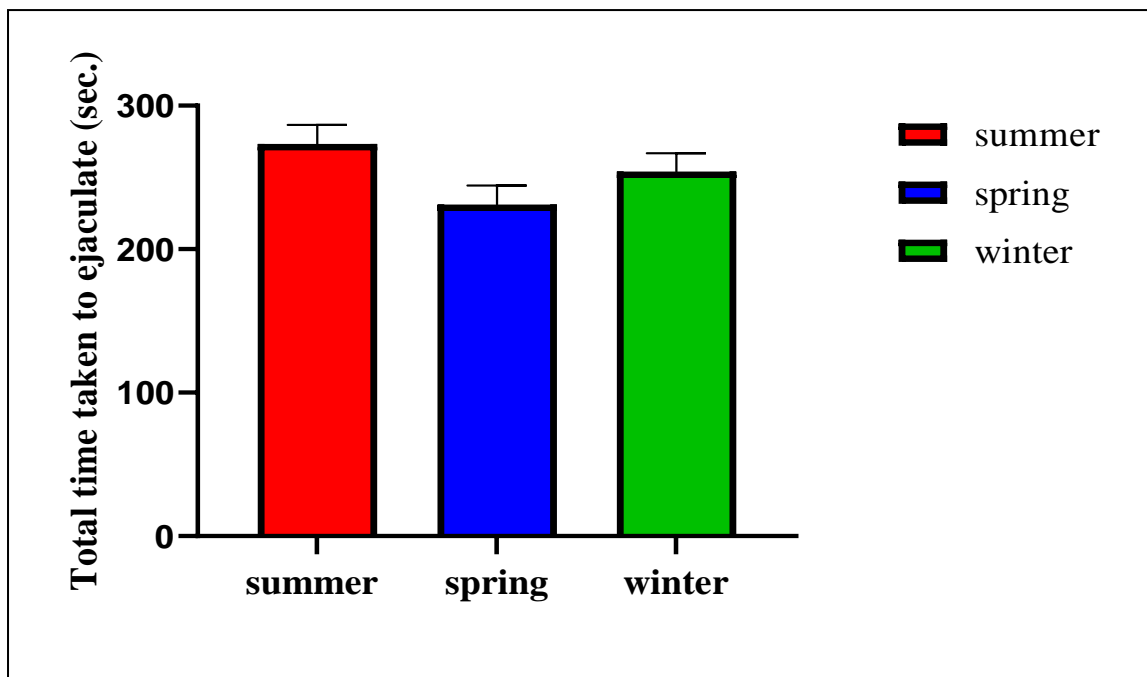


Fig. 4.20: Total time taken to ejaculate in buffalo bulls during different seasons

4.7 Effect of season on semen quality parameters of buffalo bulls

The results of seasonal variation in semen quality of Murrah buffalo bulls are presented in table 4.7 and fig. 4.21- 4.27

4.7.1 Ejaculate volume

In the present study there was no significant ($p < 0.05$) difference in the semen volume among different seasons. Ejaculate volume (mL) was highest during spring (3.04 ± 0.2) and lowest during winter season. Bhakat *et al.* (2015) and Sinha (2018) reported no significant difference in semen volume among seasons in Murrah buffalo bulls. However maximum ejaculate volume in spring season followed by dry summer, humid summer, autumn and winter seasons was obtained by Hameed *et al.* (2017) in Nili- Ravi buffalo bulls. The testicular temperature of bulls should be maintained at about 2-6 °C below their core body temperature for the production of morphologically normal and fertile spermatozoa (Waites, 1970). Heat stress affects the spermatogenesis in bulls but the volume of ejaculate is mainly contributed by the epididymal fluid and secretion of accessory sex glands and effect of season on secretion of accessory glands is not known but this increase in volume during summer might be due to increased level of testosterone in the summer month.

4.7.2 Sperm Concentration

The sperm concentration (million/mL) in Murrah buffalo bulls was 997.67 ± 97.61 in winter 1469.65 ± 91.56 in spring and 1332.6 ± 98.24 in summer. Bulls included in the study showed significantly ($p < 0.05$) higher values of sperm concentration in spring followed by summer and winter season. Javed *et al.* (2000) reported the highest sperm concentration in spring season and least in winter season in adult Nili-Ravi buffalo breeding bulls, which is in close agreement with the finding of present study. Significant seasonal influence on sperm concentration corroborates with findings of Tiwari *et al.* (2011) in Murrah bulls. However Bhakat *et al.* (2015) reported the highest sperm concentration per ml during winter season and Koonjaenak *et al.* (2007) observed no significant seasonal variation in total sperm output in Swamp buffalo. This difference in concentration might be due to differences in breed, geographical location and sexual excitement in animals.

4.7.3 Mass motility

In the present study buffalo bull showed significantly ($p < 0.05$) higher mass motility during spring (2.77 ± 0.1) season as compared to winter (2.63 ± 0.11) and summer (2.32 ± 0.15). In accordance with the results of present study Ram *et al.* (2017) reported the significantly ($P < 0.05$) higher mass motility during winter period (3.63 ± 0.09) than heat stress period (3.33 ± 0.10). But in contrary Mallick *et al.* (2016) reported no seasonal influence on mass motility in crossbred bulls. Similarly Alavi-Shoushtari *et al.* (2006) carried out study on Azarbaijani buffalo bull semen and they found higher values for mass motility in spring as compared to winter and autumn season.

4.7.4 Individual motility

In this study the individual motility (%) varied significantly ($p < 0.05$) among seasons being maximum during winter (85.17 ± 1.28) followed by spring (78.56 ± 2.62) and summer (75.88 ± 2.1). The progressive motility data obtained in the study was higher than those described by Ramadan *et al.*, (2009) for winter (56.3 ± 1.86), spring (54.2 ± 2.2) and summer (59.6 ± 1.40) in Egyptian buffalo bulls. Contrary to the findings of our study Kumar *et al.* (2020) found that the mean initial motility recorded during different seasons for Hallikar bulls was significantly higher during summer ($81.60 \pm 0.25\%$) as compared to winter ($80.20 \pm 0.33\%$). Whereas Seifi-Jamadi *et al.* (2020) reported the higher total motility in low THI group (52.36 ± 1.31) as compared to high THI group (42.23 ± 1.26) in frozen-thawed spermatozoa. The average pre and post freeze (after 30 days of freezing) motility during summer and winter season was 64.64 ± 1.37 , 34.82 ± 1.07 and 66.25 ± 1.14 , 42.32 ± 1.29 , respectively in a study by Tuli and Singh (1983) in Murrah buffalo bulls. Mitochondrial damages in sperm caused by heat stress (high THI) could be a possible reason for the reduced motility, as sperm motility is strongly dependent on the ATP produced by mitochondrial oxidative phosphorylation.

4.7.5 Viability

The sperm viability (%) was 82.70 ± 1.17 , 82.34 ± 1.46 and 77.76 ± 1.29 in winter, spring and summer season respectively. Sperm viability showed significant ($P < 0.05$) variation among the seasons and higher percent sperm viability was obtained in winter as compared to summer seasons. The results are in accordance with previous studies in Murrah buffaloes by Nitharwal *et al.* (2017) and Sinha (2018) which confirmed better survival of spermatozoa during winter season as compared to summer. In another study Alam *et al.* (2015) found that sperm viability of winter ($78.8 \pm 17.84\%$) samples was

significantly higher than that of summer (72.42 ± 14.66 %) samples in Egyptian water buffalo (*Bubalus bubalis*) bulls. Whereas Das *et al.* (2017) reported no differences in sperm viability between seasons in swamp buffalo bull's semen. Poor semen quality during summer season similar to present findings was reported by AL-Asadi *et al.* (2021) in Jonobi bulls. Due to heat stress there is excessive generation of reactive oxygen species (ROS) and depletion of antioxidant potential, which leads to lipid peroxidation in sperm plasma membrane; this might be reason for decreased viability during summer.

4.7.6 Acrosome integrity

The mean percent sperm acrosome integrity in the present study was 83.1 ± 1.02 in winter; 4.96 ± 1.09 in spring and 81.43 ± 1.17 in summer. In this study season showed no significant effect on sperm acrosome integrity. However, findings of our study are in accordance with Parmar *et al.* (2019) in Jaffarabadi breeding bulls and Mallick *et al.* (2016) in Karan Fries bulls. Baruti *et al.* (2019) showed a significant effect of season on acrosome integrity being highest in winter and least during summer in swamp buffalo bulls. Similarly Soren *et al.* (2016) reported a significant ($p < 0.05$) difference in acrosome integrity between hot humid and spring season. Whereas Valeanu *et al.* (2014) reported no significant seasonal differences in acrosome damage status.

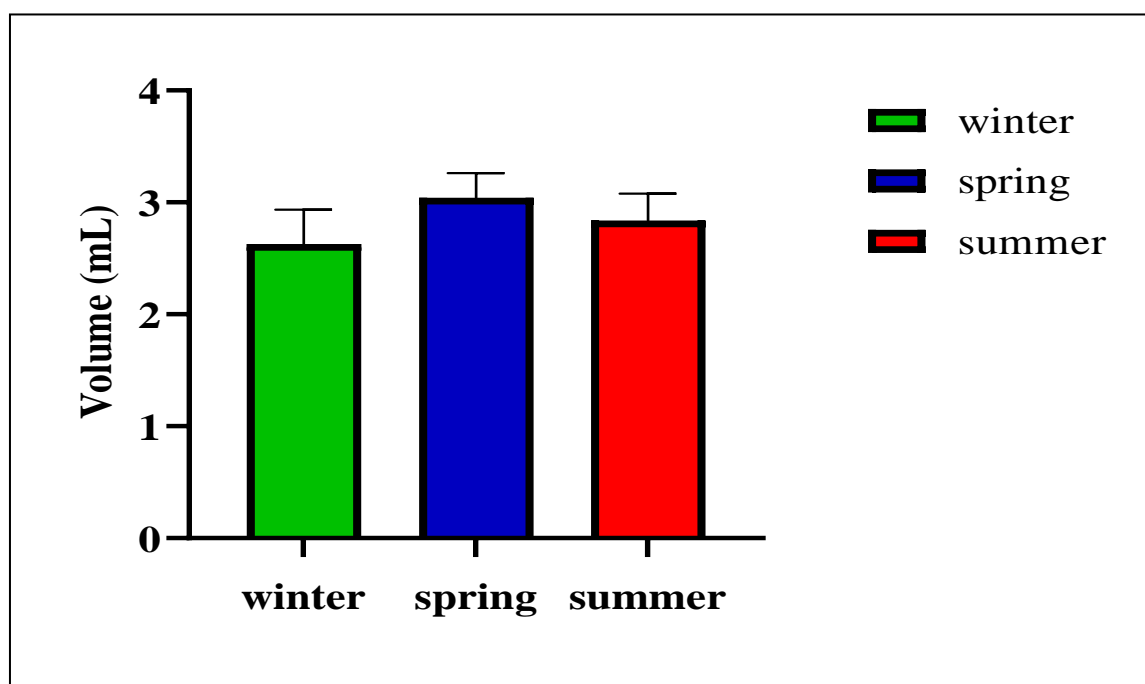
4.7.7 Hypo-osmotic swelling test (HOST) Reacted Spermatozoa

Hypo-osmotic swelling test is done to evaluate the sperm membrane integrity. A significant ($P < 0.05$) seasonal variation in hypo-osmotic swelling test (HOST) reacted spermatozoa was observed in the present investigation. The highest HOST positive (%) was observed in spring lowest during summer (74.13 ± 1.09 Vs. 66.19 ± 2.33). Mandal *et al.* (2003) investigated the motion characteristics of Murrah buffalo bull spermatozoa in different seasons and their association with plasma membrane functional integrity. A considerably highest HOS reactivity (%) of spermatozoa (65.04 ± 0.05) found during the winter season and the % HOS-positive spermatozoa did not differ significantly between the summer (54.5 ± 0.06) and rainy (55.4 ± 0.05) seasons. Similarly Khawaskar *et al.* (2012) also reported that the winter is the best time for Surti buffalo bulls to produce spermatozoa with a higher proportion of intact plasma membrane. Decrease in host positive spermatozoa in summer season might be due to the damage of sperm plasma membrane due to overproduction of ROS (reactive oxygen species) and due to this oxidative damage the spermatozoa plasma membrane loses its fluidity and structure that related to poor sperm fertility and motility.

Table 4.7: Effect of season on semen quality parameters (Mean± SE) of buffalo bulls

Semen quality attributes	Winter	Spring	Summer
Volume (mL)	2.63±0.31	3.04±0.22	2.84±0.24
Concentration (million/mL)	997.67 ^a ±97.61	1469.65 ^b ±91.56	1332.6 ^b ±98.24
Mass motility(score 0-5)	2.63 ^{ab} ±0.11	2.77 ^a ±0.1	2.32 ^b ±0.15
Individual motility (%)	85.17 ^a ±1.28	78.56 ^{ab} ±2.62	75.88 ^b ±2.21
Viability (%)	82.70 ^a ±1.17	82.34 ^a ±1.46	77.76 ^b ±1.29
Acrosome integrity (%)	83.1±1.02	84.96±1.09	81.43±1.17
HOST positive (%)	72 ^a ±1.2	74.13 ^a ±1.09	66.19 ^b ±2.33

Means bearing different superscripts in a row differ significantly (p<0.05)

**Fig. 4.21: Ejaculate volume in buffalo bulls during different seasons**

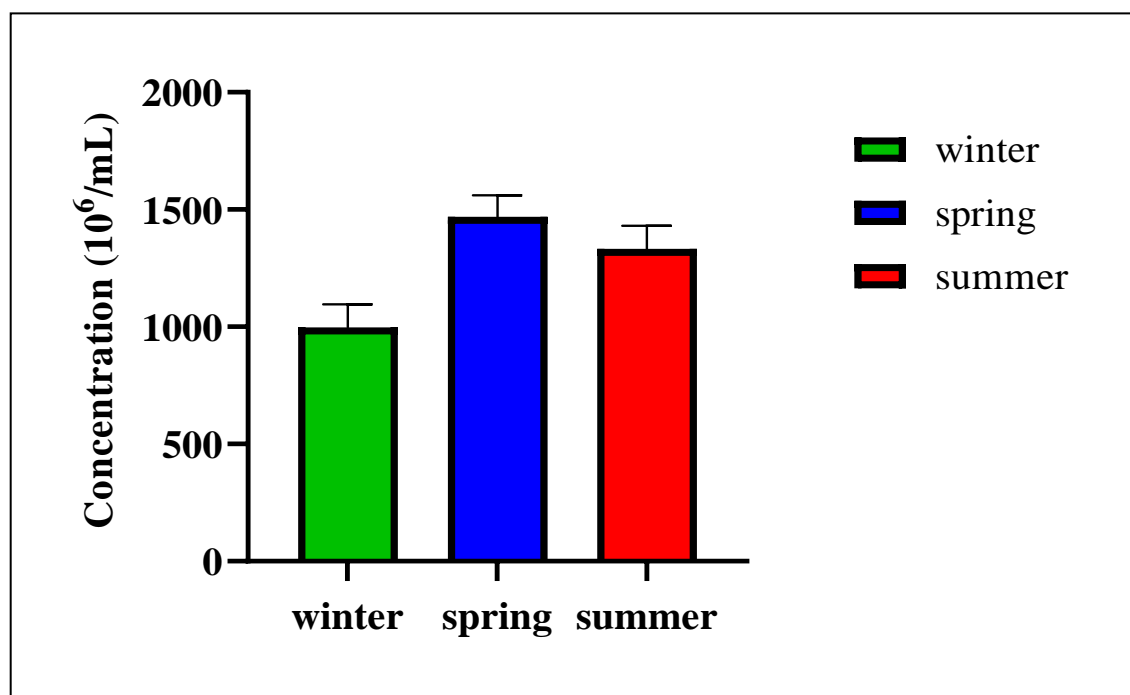


Fig. 4.22: Sperm concentration in buffalo bulls during different seasons

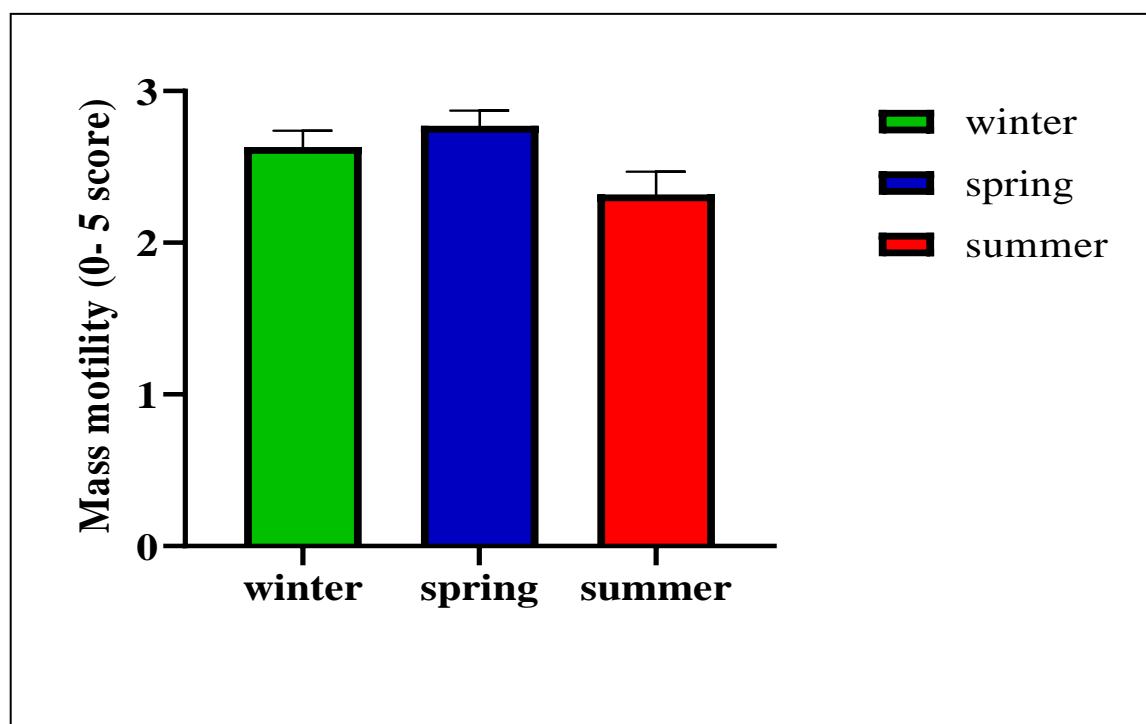


Fig. 4.23: Mass motility of semen in buffalo bulls during different seasons

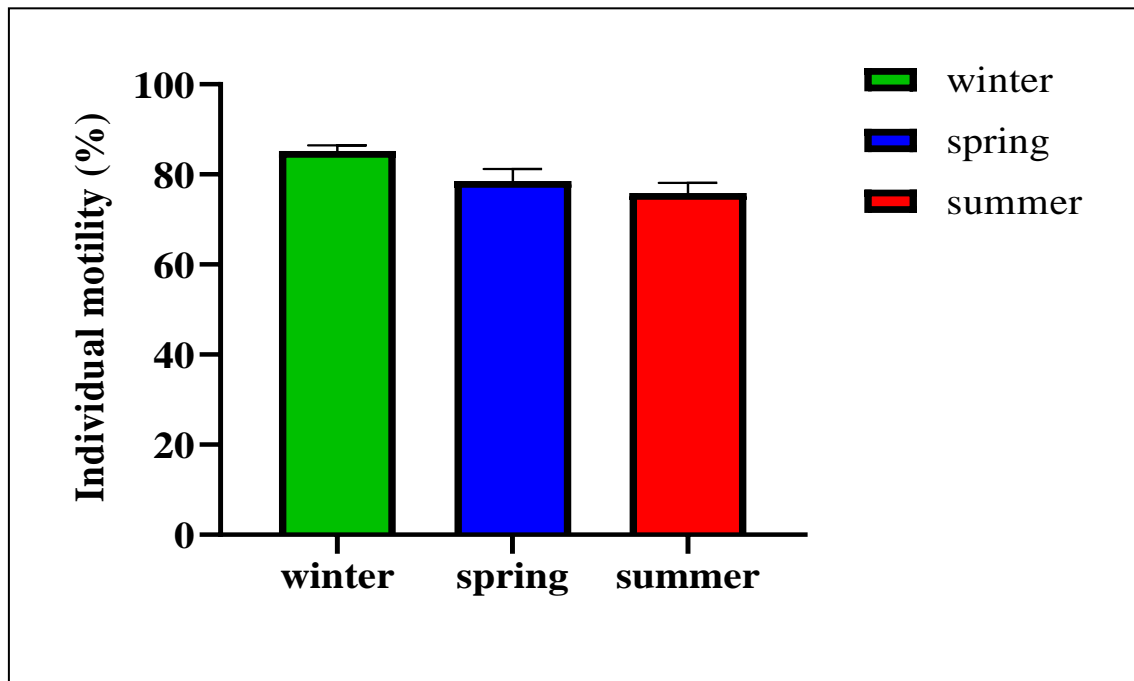


Fig. 4.24: Individual motility of spermatozoa in buffalo bulls during different seasons

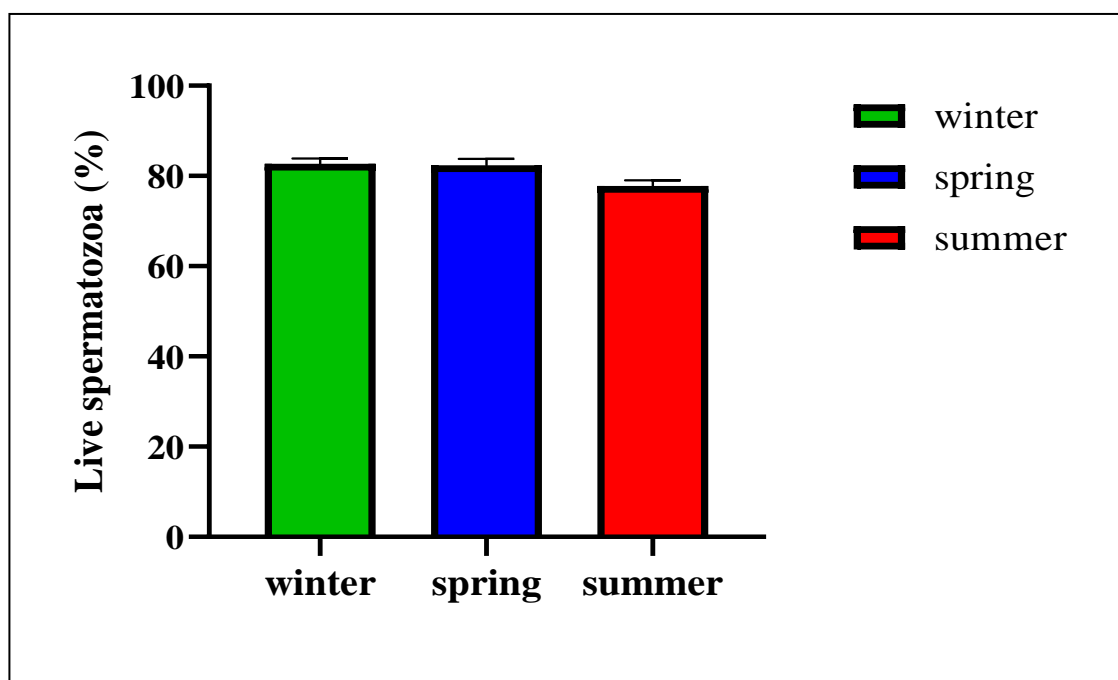


Fig. 4.25: Live spermatozoa in buffalo bulls during different seasons

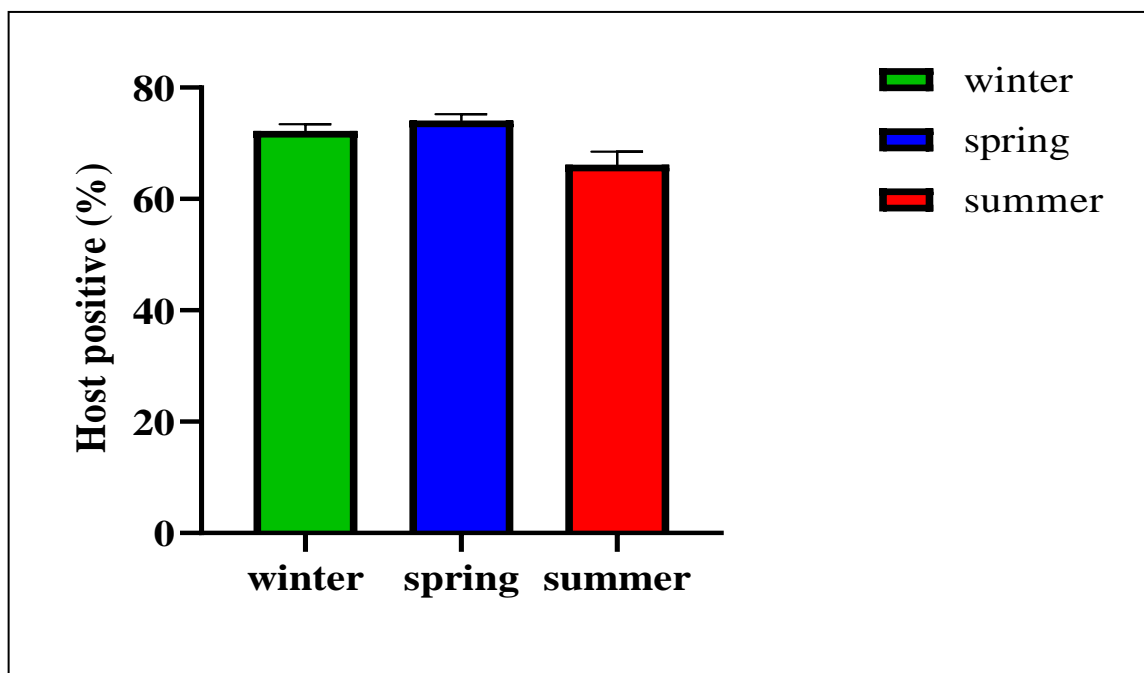


Fig. 4.26: HOST positive spermatozoa in buffalo bulls during different seasons

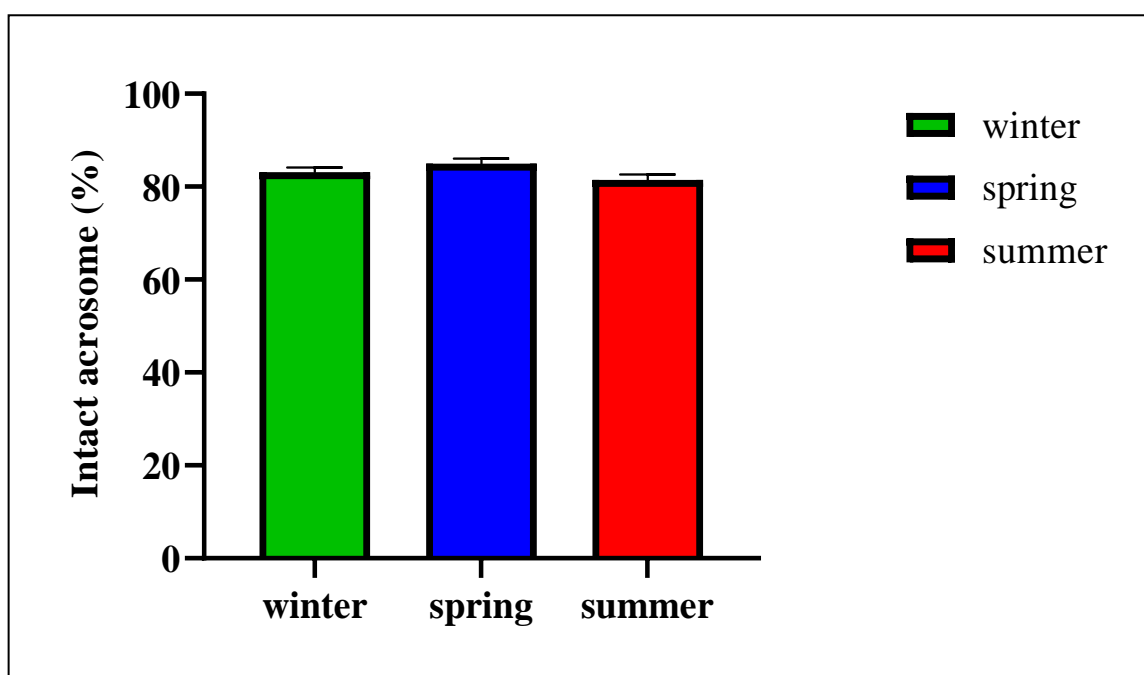


Fig. 4.27: Intact spermatozoa in buffalo bulls during different seasons

4.7.8 Effect of season on sperm abnormalities in buffalo bulls

The results of seasonal variation in sperm abnormalities of Murrah buffalo bulls are presented in table 4.8.

In this study sperm abnormalities were 10.74 ± 0.3 for winter, 9.46 ± 0.28 for spring and 14.46 ± 0.4 for summer, summer season showed highest sperm abnormalities. Koonjaenak *et al.* (2007) studied overall total mean percentages of sperm abnormalities in buffalo bull spermatozoa were $< 15\%$, with the rainy season accounting for ($13.7 \pm 0.5\%$), winter ($12.4 \pm 0.5\%$), and summer ($10.7 \pm 0.5\%$). Summer had the highest proportion of morphologically normal spermatozoa and winter had the lowest ($P < 0.05$). In contrary de Castro *et al.* (2017) total 30 samples were collected per bull, namely 10 ejaculates in the rainy season and 20 ejaculates in the non-rainy season. The evaluation of the semen samples was performed on fresh semen, immediately after collection $2 \pm 3.9\%$, total defects $20.2 \pm 5.3\%$, whereas in then on rainy season, major defects were $20.8 \pm 9.9\%$, minor defects were $27.5 \pm 6.3\%$, and total defects were $48.3 \pm 9.3\%$. It was concluded that the ideal period for collection of buffalo ejaculates was the months of the rainy season in this period a better quality ejaculates of buffaloes were obtained. During heat stress the normal tissue maintenance and regenerative activities are compromised and increases cellular death. Increased morphological sperm abnormalities when animals were exposed to high THI are assumed to be related to defective chromatin condensation during the acrosome and Golgi phase of spermiogenesis as spermatozoa are known to be more vulnerable to heat stress when these cells are not completely protaminated.

Table 4.8: Effect of season on sperm abnormalities (Mean \pm SE) in buffalo bulls

Sperm morphology (%)	Winter	Spring	Summer
Head Abnormality	$2.47^b \pm 0.12$	$1.98^a \pm 0.09$	$3.85^c \pm 0.77$
Mid Piece Abnormality	$3.49^a \pm 0.16$	$3.22^a \pm 0.12$	$4.92^b \pm 0.15$
Tail Abnormality	$4.78^b \pm 0.1$	$4.25^a \pm 0.13$	$5.69^c \pm 0.14$
Total Abnormality	$10.74^b \pm 0.3$	$9.46^a \pm 0.28$	$14.46^c \pm 0.4$

Means bearing different superscripts in a row differ significantly ($p < 0.05$)

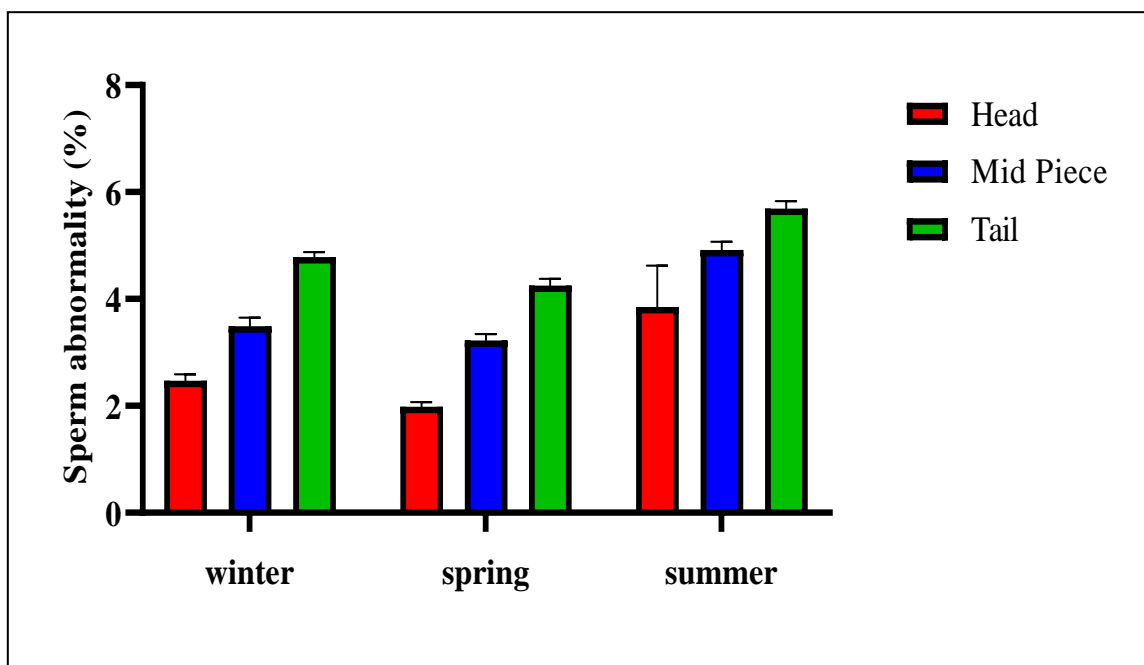


Fig. 4.28: Sperm abnormalities in buffalo bulls during different seasons

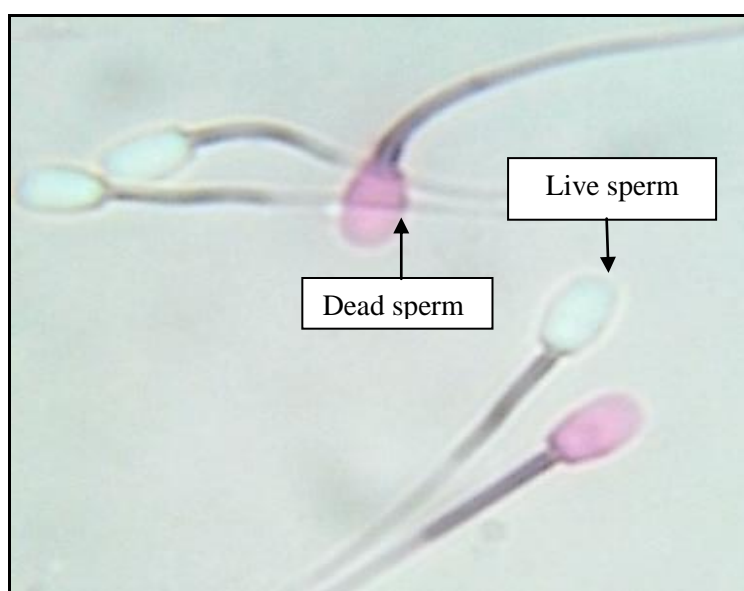


Plate no. 6: Live and dead spermatozoa

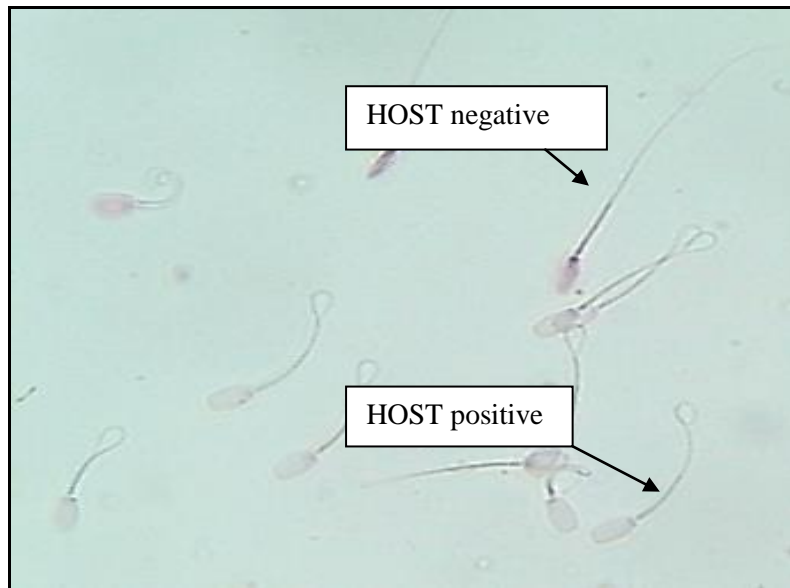


Plate no. 7: HOST reacted spermatozoa

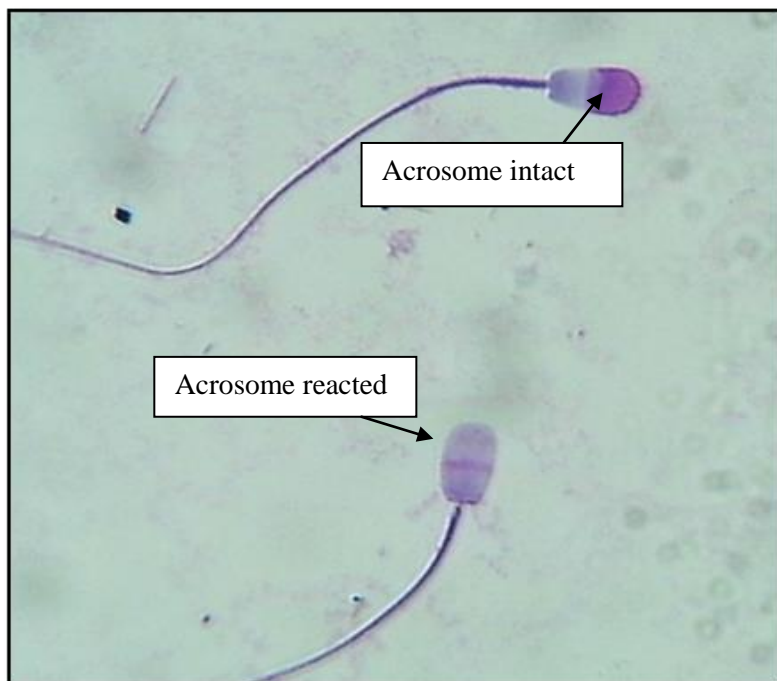


Plate no. 8: Acrosome reacted spermatozoa

CHAPTER -5

Summary and Conclusions

SUMMARY AND CONCLUSION

The present investigation was carried out on fourteen mature Murrah buffalo bulls of around 3-4 years age which were maintained at Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal (Haryana). Depending upon the climatic conditions, the experimental period was divided into three seasons viz., winter (15 December to 15 February), spring (16 February to 30 April), summer (1 May to 30 June). The meteorological data like temperature and relative humidity during the study period was retrieved from Central Soil Salinity Research Institute (CSSRI), Karnal, India. Temperature humidity index (THI) was calculated with the help of aforementioned variables. In this study, effect of winter, spring and summer season on animals physiological responses, body surface temperature, sexual behavior, endocrinological profile and semen quality was assessed on randomly selected Murrah buffalo bulls. Physiological response like temperature was recorded with the help of digital thermometer and respiration rate was recorded by counting the movement of flank per minute. Infrared thermal imaging technique was employed to record the surface temperature of different body surfaces viz., eye, forehead, flank, rump and scrotal surface. The infrared thermograms were taken with the help of hand held FLIR i7 camera at a distance of 1 meter from the animal and images were analyzed with the help of FLIR tools analysis software. Thermal imaging of scrotum was done at different points i.e. Upper surface/proximal pole temperature (PPT), mid surface/ mid pole temperature (MPT) and lower surface/distal pole temperature (DPT). The temperature gradient (TG) was calculated by the difference between the temperature of upper and lower scrotal surface. Semen was collected twice a week using buffalo bull specific Artificial Vagina (IMV Technologies, France) method, with standard semen collection procedure in the morning hours at 7:30 AM. Sexual behavior of breeding bulls was recorded at the time of semen collection. Blood samples for hormonal analysis were collected on the same day of semen collection from jugular vein and serum was separated. The data generated from experiments were subjected to statistical analysis by one-way analysis of variance (ANOVA) using SPSS software and means were compared using Duncan's multiple range tests to draw scientific inferences. The summary of the present investigation has been presented under the following headings:

Summary and Conclusions

Seasonal variation in Meteorological variables and temperature humidity index (THI)

- The mean values for ambient temperature (°C) recorded during winter, spring and summer season was 9.43 ± 0.43 , 17.99 ± 0.52 and 26.6 ± 0.29 in the morning and 19.56 ± 0.63 , 32.19 ± 0.58 and 34.87 ± 0.52 in the afternoon, respectively. The ambient temperature varied ($p < 0.05$) among different seasons, it was lowest in winter and highest in summer season.
- The relative humidity (%) during winter, spring and summer season was 98.47 ± 0.48 , 75.75 ± 2.75 and 73.52 ± 1.55 for morning hours and 78.66 ± 1.8 , 41.69 ± 2.78 and 45.82 ± 1.87 in the afternoon. It was higher ($p < 0.05$) in winter season as compared to spring and summer.
- The value of THI was highly variable among different seasons it was 58.01 ± 0.75 in winter; 72.36 ± 0.5 in spring and 80.79 ± 0.38 in summer season. It was maximum during summer (80.79 ± 0.38) followed by spring (72.36 ± 0.5) and winter (58.01 ± 0.75) season.

Physiological responses in Murrah bulls during different season

- The mean values for rectal temperatures (°C) in morning hours during winter, spring and summer seasons were 36.91 ± 0.05 , 37.27 ± 0.05 and 37.58 ± 0.05 , respectively. Value of rectal temperatures during morning hours was higher ($p < 0.05$) in summer season as compared to spring and winter season. Similarly for afternoon during winter, spring and summer season were 37.19 ± 0.40 , 37.83 ± 0.03 and 38.16 ± 0.06 , respectively. Higher values for rectal temperature in the afternoon were during summer and spring as compared to winter season.
- The respiration rate (breaths/ minute) was 11.88 ± 0.12 in winter; 15.56 ± 0.17 in spring and 24.38 ± 0.42 in summer during morning hours and 14.25 ± 0.19 in winter, 19.78 ± 0.26 in spring 29.74 ± 0.55 in summer during afternoon hours. An increase ($p < 0.05$) in respiration rate was observed in summer season as compared to spring and winter season

Body surface temperature (°C) in Murrah bulls during different season.

- The surface temperature (°C) recorded in the morning hours was 33.51 ± 0.52 , 34.2 ± 0.21 and 36.77 ± 0.14 for eye; 25.4 ± 0.58 , 27.58 ± 0.57 and 34.4 ± 0.36 for forehead; 26.98 ± 0.42 ,

29.47±0.5 and 34.79±0.26 for flank region and 26.86±0.28, 28.43±0.54 and 34.9±0.28 for rump region during winter, spring and summer season respectively.

- The temperature recorded during afternoon was 36.17±0.24, 36.17±0.24 and 36.17±0.24 for eye; 32.6±0.36, 35.42±0.36 and 39.1±0.23 for forehead; 34.14±0.4, 35.44±0.43 and 38.56±0.25 for flank and 35±1.34, 36.33±0.35 and 39±0.23 for rump region during winter, spring and summer season, respectively.
- The scrotal surface temperature (°C) at upper, mid and lower point/part during morning (6-7 AM) hours was 32.79±0.24, 30.79±0.30 and 27.18±0.18, respectively in winter; 33.51±0.17, 31.61±0.30 and 26.88±0.23 in spring and 35.86±0.20, 34.0±0.21 and 30.49±0.32, respectively during summer season. During afternoon (2-3 PM) hours it was 34.25±0.27, 32.68±0.32 and 29.91±0.36 during winter; 35.6±0.19, 34.1±0.19 and 31.05±0.35, respectively during spring and 37.7±0.17, 36.2±0.20 and 33.55±0.25, respectively during summer season. The scrotal temperature varied ($p<0.05$) among the different location across the season, lowest temperature was in winter followed by spring and summer season.

Endocrinological profile of Murrah buffalo bulls during different season

- The mean TSH (μ IU/mL) concentration during different seasons was higher ($P<0.05$) in winter (0.020) and spring (0.016) season as compared to summer and the concentration of T3 (ng/mL) was 4.98±0.21 in winter, 4.28±0.14 in spring and 1.79±0.31 during summer season.
- Higher ($P<0.05$) value of T3 was in winter season and least during summer season. Similarly the mean concentration of T4 (ng/mL) hormone was 54.54±1.77 in winter; 53.24±1.88 in spring and 37.71±1.05 in summer with lower ($P<0.05$) value in summer season and higher during winter and spring season was obtained.
- The mean of cortisol (ng/ml) concentration in the present study was 4.59±1.21 during winter; 3.41±0.62 in spring and 4.24±0.42 in summer season. The concentration of cortisol was higher during winter months.
- The testosterone (ng/mL) concentration was significantly higher ($P<0.05$) during summer (2.41±0.37) as compared to spring (1.46±0.18) and winter (1.09±0.16).

Sexual behavior of Murrah buffalo bulls during different season

- The mean of reaction time of bulls was 264.57 ± 13.91 in summer; 225.07 ± 12.96 in spring; 248.57 ± 12.85 in winter. The reaction time did not vary among the season whereas longer reaction time was longer in summer than winter and was least during spring season
- The mean values for libido score (LS) of Murrah buffalo bulls was significantly higher ($P < 0.05$) during the spring (38 ± 1.72) and winter (36.71 ± 1.48) season as compared to summer (29.17 ± 3.04).
- The Murrah buffalo bulls included in the study showed significantly ($p < 0.05$) higher values for total time taken to ejaculate during summer (273.36 ± 13.15) as compared to spring (231.21 ± 13) and winter (254.21 ± 12.67).

Effect of season on semen quality parameters of Murrah buffalo bulls

- In the present study there was no significant ($p < 0.05$) difference in the semen volume among different seasons. Ejaculate volume (mL) was highest during spring (3.04 ± 0.2) and lowest during winter season.
- The sperm concentration (million/mL) in Murrah buffalo bulls was 997.67 ± 97.61 in winter 1469.65 ± 91.56 in spring and 1332.6 ± 98.24 in summer. Bulls included in the study showed significantly ($p < 0.05$) higher values of sperm concentration in spring followed by summer and winter season.
- Murrah buffalo bull showed significantly ($p < 0.05$) higher Mass motility during spring (2.77 ± 0.1) season as compared to winter (2.63 ± 0.11) and summer (2.32 ± 0.15).
- The individual motility (%) varied significantly ($p < 0.05$) among season being maximum during winter (85.17 ± 1.28) followed by spring (78.56 ± 2.62) and summer (75.88 ± 21).
- The Mean \pm SE of sperm viability (%) was 82.70 ± 1.17 , 82.34 ± 1.46 and 77.76 ± 1.29 in winter, spring and summer season respectively. Sperm viability showed significant ($P < 0.05$) variation among the seasons and higher percent sperm viability was obtained in winter as compared to summer seasons.

- The mean percent sperm acrosome integrity in the present study was 83.1 ± 1.02 in winter; 4.96 ± 1.09 in spring and 81.43 ± 1.17 in summer. In this study season showed no significant effect on sperm acrosome integrity.
- Hypo-osmotic swelling test is done to evaluate the sperm membrane integrity. A significant ($P < 0.05$) seasonal variation in hypo-osmotic swelling test (HOST) reacted spermatozoa was observed in the present investigation. The highest HOST positive (%) was observed in spring lowest during summer (74.13 ± 1.09 Vs. 66.19 ± 2.33)
- In these study sperm abnormalities in semen of buffalo bulls in this study was 10.74 ± 0.3 for winter, 9.46 ± 0.28 for spring and 14.46 ± 0.4 for summer, summer season showed highest sperm abnormalities

Conclusions

From this study the following conclusions can be drawn

- Rectal temperature and respiration rate of buffalo bulls influenced by Season, this value were significantly higher during summer season as compared to cooler months (winter and spring).
- Activity of thyroid gland reduced (low level of T3, T4 and TSH) during summer compared to winter and spring seasons, whereas cortisol level did not differ significantly among the seasons. However, testosterone concentration found to be significantly higher in summer compared to spring and winter.
- The buffalo bulls showed higher reaction time and total time taken for ejaculation in summer as compared to cooler months, whereas the libido score was significantly higher in spring season.
- Scrotal surface temperature gradient was significantly higher in the morning hour during spring season than winter and summer, similarly most of the semen quality parameters (sperm concentration, mass motility, individual motility and HOST reacted spermatozoa) were found to be better in spring season except sperm acrosome integrity.

Summary and Conclusions

- Overall it can be concluded that the physiological responses, testicular temperature, hormonal profile, sexual behavior and semen quality of Murrah buffalo bulls were significantly influenced by seasons of the year.

Recommendation

- Seasonal stress on buffalo bulls can be overcome through recommended management practices to improve the overall reproductive performance.

Bibliography

BIBLIOGRAPHY

- Ahirwar, M.K., Kataktaaware, M.A., Pushpadass, H.A., Jeyakumar, S., Jash, S., Nazar, S., Kastelic, J.P. and Ramesha, K.P. (2018). Scrotal infrared digital thermography predicts effects of thermal stress on buffalo (*Bubalus bubalis*) semen. *Journal of thermal biology*, 78: 51-57.
- Ahmad, M., Asmat, M.T. and Rehman, N.U. (2005). Relationship of testicular size and libido to age and season in Sahiwal bulls. *Pakistan Veterinary Journal*, 25(2): 67-70.
- Aitken, R.J. and Curry, B.J. (2011). Redox regulation of human sperm function: from the physiological control of sperm capacitation to the etiology of infertility and DNA damage in the germ line. *Antioxidants & redox signaling*, 14(3): 367-381.
- Alam, S.S., El Makawy, A.I., Tohamy, A.A. and Abd Elrahman, M.M. (2015). Effect of seasonal variations on semen quality and fertility of Egyptian Water Buffalo (*Bubalus bubalis*) bulls. *Research journal of pharmaceutical biological and chemical sciences*, 6(6): 1059-1069.
- AL-Asadi, F.A., Kassim, W.Y. and Hassan, A.F. (2021). Effect of Season and Age on Semen Characteristics and Physiological Changes in the Testis of Jonobi Bull Breed in Iraq. *Annals of the Romanian Society for Cell Biology*, 2535-2542.
- Alavi-Shoushtari, S.M. and Abazadeh-Habashi, B. (2006). Seasonal variation in the characteristics of the Azarbaijani buffalo (*Bubalus bubalis*) semen. *Iranian Journal of Veterinary Research*, 7(1): 49-54.
- Amann, R.P. and Almquist, J.O. (1976). Bull management to maximize sperm output. In *Proceedings of Technical Conference on Artificial Insemination and Reproduction*.
- Anderson, J. (1945). The semen of animals and its use for artificial insemination. *Tech. Comm. Imp. Bur. Anim. Breeding and Genetics*, Edinburgh.

Bibliography

- Anzar, M., Ahmad, M., Nazir, M., Ahmad, N. and Shah, I.H. (1993). Selection of buffalo bulls: Sexual behavior and its relationship to semen production and fertility. *Theriogenology*, 40(6): 1187-1198.
- Badreldin, A.L. and Ghany, M.A. (1954). Species and breed differences in the thermal reaction mechanism. *The Journal of Agricultural Science*, 44(2): 160-164.
- Barnabe, V.H., Barnabe, R.C., Oliveira, C.A., Gambarini, M.L., Guido, M.C., Valentim, R. (1995). Testosterone concentration in blood serum of buffalo bulls during 24 hours in winter and summer seasons. *Braz. J. vet. Res. anim. Sei*, 32(3): 191-193.
- Barros, D.V., Silva, L.K.X., de Brito Lourenço, J., da Silva, A.O.A., e Silva, A.G.M., Franco, I.M., Oliveira, C.M.C., Tholon, P., Martorano, L.G. and Garcia, A.R. (2015). Evaluation of thermal comfort, physiological, hematological, and seminal features of buffalo bulls in an artificial insemination station in a tropical environment. *Tropical animal health and production*, 47(5): 805-813.
- Barth, A.D. and Bowman, P.A. (1994). The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. *The Canadian Veterinary Journal*, 35(2): 93.
- Bartke, A., Klemcke, H. and Matt, K. (1986). Effects of physiological and abnormally elevated prolactin levels on the pituitary-testicular axis. *Medical biology*, 63(5-6): 264-272.
- Baruti, M., Deka, B.C., Tamuly, S., Bhuyan, M., Borah, P., Das, G.C., Deka, N., Deka, R., Biswas, R.K. and Sinha, S. (2019). Seasonal effect on seminal constituents of swamp buffalo (*Bubalus carabanesis*) in the foot hill of Eastern Himalaya. *Biological Rhythm Research*, 1-12.
- Basic Animal Husbandry Statistics. (2019). Ministry of Fisheries Animal Husbandry and Dairying. Annual report, 2018-19.
- Basic Animal Husbandry Statistics. (2020). Ministry of Fisheries Animal Husbandry and Dairying. Annual report, 2020-21.
- Bhakat, M., Mohanty, T.K., Gupta, A.K. and Abdullah, M. (2014) Effect of season on semen quality of crossbred (Karan Fries) bulls. *Adv. Anim. Vet. Sci.*, 2(11): 632-637.

- Bhakat, M., Mohanty, T.K., Gupta, A.K., Prasad, S., Chakravarty, A.K. and Khan, H.M. (2015). Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin*, 34(1): 100-112.
- Bhakat, M., Mohanty, T.K., Raina, V.S., Gupta, A.K., Khan, H.M., Mahapatra, R.K. and Sarkar, M. (2011) Effect of age and season on semen quality parameters in Sahiwal bulls. *Tropical animal health and production*, 43(6): 1161-1168.
- Bhan, C., Singh, S.V., Hooda, O.K., Upadhyay, R.C. and Beenam, B. (2013). Influence of temperature variability on physiological, hematological and biochemical profiles of growing and adult Karan Fries cattle. *Indian Journal of Animal Sciences*, 83(10): 1090-1096.
- Blazquez, N. B., Mallard, G. J. and Wedd, S. R. (1988). Sweat glands of the scrotum of the bull. *Reproduction*, 83(2): 673-677.
- Boni, R. (2019). Heat stress, a serious threat to reproductive function in animals and humans. *Molecular reproduction and development*, 86(10): 1307-1323.
- Brandon, R.N. (2014). *Adaptation and environment*. Princeton University Press
- Brito L.F.C., Silva A.E.D.F., Barbosa R.T., and Kastelic, J.P. (2004). Testicular thermoregulation in *Bos indicus*, crossbred and *Bos taurus* bulls: relationship with scrotal, testicular vascular cone and testicular morphology, and effects on semen quality and sperm production. *Theriogenology*, 61(2-3): 511-28.
- Brito, L. F., Silva, A. E., Barbosa, R. T., Unanian, M. M., and Kastelic, J. P. (2003). Effects of scrotal insulation on sperm production, semen quality, and testicular echotexture in *Bos indicus* and *Bos indicus*×*Bos taurus* bulls. *Animal reproduction science*, 79(1-2): 1-15.
- Brito, L.F.C., Silva, A.E.D.F., Rodrigues, L.H., Vieira, F.V., Deragon, L.A.G. and Kastelic, J.P (2002). Effect of age and genetic group on characteristics of the scrotum, testes and testicular vascular cones, and on sperm production and semen quality in AI bulls in Brazil. *Theriogenology*, 58(6): 1175-1186.
- Campbell, R.C., Hancock, J.L. and Rothschild, L. (1953). Counting live and dead bull spermatozoa. *Journal of Experimental Biology*, 30(1): 44-49.

Bibliography

- Chacur, M.G.M., Mizusaki, K.T., Gabriel Filho, L.R.A., Oba, E. and Ramos, A.A. (2013). Seasonal Effects on Semen and Testosterone in Zebu and Taurine Bulls. *Acta Scientiae Veterinariae*, 41(1): 1-5.
- Chaudhary, S.S., Singh, V.K., Upadhyay, R.C., Puri, G., Odedara, A.B. and Patel, P.A. (2015). Evaluation of physiological and biochemical responses in different seasons in Surti buffaloes. *Veterinary world*, 8(6):727-731.
- Chenoweth, P.J. (1981). Libido and mating behavior in bulls, boars and rams: A review. *Theriogenology*, 16(2): 155-177.
- Chenoweth, P.J. (1983). Sexual behavior of the bull: a review. *Journal of Dairy Science*, 66(1): 173-179.
- Correa, J. R., and Zavos, P. M. (1994). The hypo-osmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen- thawed bovine sperm membrane. *Theriogenology*, 42: 351-360
- Cunningham J.G. (2008) Tratado de Fisiologia Veterinária. 4^{ed}. Guanabara Koogan, Rio de Janeiro.
- Dandage S. D. (2009). Estimates of thermal load and heat exchange in cattle and buffaloes. *M.V.Sc. Thesis*. NDRI, Deemed University, Karnal (Haryana), India.
- Das, G.C., Das, P.K., Deori, S., Mazumdar, H., Bhattacharyya, B.N. and Phookan, A. (2017). Seasonal variation in the characteristics of the swamp buffalo semen of northeast India. *Buffalo Bulletin*, 36(1): 215-220.
- Das, S.K., Upadhyay, R.C. and Madan, M.L. (1997). Changes in skin temperature and physiological reactions in Murrah buffalo during solar exposure in summer. *Asian-Australasian Journal of Animal Sciences*, 10(5): 478-483
- Dayal, S., Dey, A., Pandian, S.J., Gupta, J.J., Chandran, P.C. and Ali, I. (2017). Effect of seasonal variation on physiological parameters in Murrah buffaloes. *Indian Journal of Animal Sciences*, 87(8): 965-967.
- de Castro, S.R.S., Morini, A.C., da Silva, A.D.S.L., Neves, K.A.L., Minervino, A.H.H., de Moura Meneses, A.A. and Vale W.G. (2017). Influence of Seasonality on Buffalo Semen. *Animal and Veterinary Sciences*, 5(3): 45-51

- Deka, R., Patel, J.B., Srivastava, A.K., Prajapati, K.B., Patel, J. and Rathod, B.S. (2019) Seasonal effect on sexual behaviour of Kankrej bull. *Indian J. Dairy Sci*, 72(5): 506-513.
- Diebold, L. and Chandel, N.S. (2016). Mitochondrial ROS regulation of proliferating cells. *Free Radical Biology and Medicine*, 100: 86-93.
- 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? *J. Dairy Sci.*, 92: 109-16.
- Dixit, N.K., Agarwal, S.P., Agarwal, V.K. and Dwaraknath, P.K. (1984). Seasonal variations in serum levels of thyroid hormones and their relation with seminal quality and libido in buffalo bulls. *Theriogenology*, 22(5): 497-507.
- El Nouty, F.D. and Hassan, G.A. (1983). Thyroid hormone status and water metabolism in Hereford cows exposed to high ambient temperature and water deprivation. *Indian Journal of Animal Sciences*, 53(8): 807-812
- Elrabie, K.A., Raina, V.S., Gupta, A.K. and Mohanty, T.K. (2008). Effect of semen collection floor on sexual behaviour and semen quality of Sahiwal bulls. *Pak. J. Agri. Sci*, 45(2): 201-205
- Farooq, U., Samad, H.A., Shehzad, F. and Qayyum, A. (2010). Physiological responses of cattle to heat stress. *World Appl. Sci. J*, 8: 38-43.
- Ferro F, CavalcantiNeto C, Toledo Filho MR, Ferri S, Montaldo Y. C. (2010). Effect of heat stress on the reproductive performance of dairy cows. *Green Magazine*. 5: 01-25
- Fregly, M.J. (1989). Activity of the hypothalamic-pituitary-thyroid axis during exposure to cold. *Pharmacology & therapeutics*, 41(1-2): 85-142.
- Garcia A.R. (2006) Influência de fatores ambientais sobre as características reprodutivas de búfalos do rio (*Bubalus bubalis*). *Rev Ciênc Agrár Belém*, 45: 1–13.
- Garcia-Oliveros, L.N., de Arruda, R.P., Batissaco, L., Gonzaga, V.H.G., Nogueira, V.J.M., Florez-Rodriguez, S.A., Almeida, F.D.S., Alves, M.B.R., Pinto, S.C.C., Nichi, M. and Losano, J.D.D.A. (2020). Heat stress effects on bovine sperm cells: a chronological approach to early findings. *International journal of biometeorology*, 64: 1367-1378.

Bibliography

- Gaughan, J.B., Mader, T.L., Holt, S.M. and Lisle, A. (2008). A new heat load index for feedlot cattle. *Journal of Animal Science*, 86(1): 226-234.
- Gloster, J., Ebert, K., Gubbins, S., Bashiruddin, J. and Paton, D.J. (2011). Normal variation in thermal radiated temperature in cattle: implications for foot-and-mouth disease detection. *BMC veterinary research*, 7(1): 1-10.
- Godfrey, R.W., Lunstra, D.D., Jenkins, T.G., Berardinelli, J.G., Guthrie, M.J., Neuendorff, D.A., Long, C.R. and Randel, R.D. (1990). Effect of season and location on semen quality and serum concentrations of luteinizing hormone and testosterone in Brahman and Hereford bulls. *Journal of animal science*, 68(3): 734-749.
- Goswami, S.B. (1962). The effect of administration of thyroxine and PMS hormones on blood corpuscular counts of buffalo bulls (*Bubalus bubalis*). *The Indian veterinary journal*, 42(9): 670-674.
- Greenspan F.S. (2006). Basic and Clinical Endocrinology. 7th edn. McGraw-Hill, Porto Alegre.
- Hafez, E. S. E. and Hafez, B. (2013). *Reproduction in Farm Animals* 7th edn, 293–397 (Lippincott Williams and Wilkins).
- Hameed, S., Masood, S., Zaneb, H., Khan, M.S., Younis, M., Avais, M. and Khan, M.U.R. (2017). Semen characteristics as influenced by seasonal and climatic variations in Nili-Ravi buffalo breeding bulls. *Journal of Animal & Plant Sciences*, 27(6): 1750-1757
- Hancock, J.L. (1952). The morphology of bull spermatozoa. *Journal of Experimental Biology*, 29(3): 445-453.
- Hansen, P. J. (2009). Effects of heat stress on mammalian reproduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1534): 3341-3350.
- Hees H, Leiser R, Kohler T and Wrobel K. (1984). Vascular morphology of the bovine spermatic cord and testis. Light- and scanning electron-microscopic studies on the testicular artery and pampiniformplexus. *Cell and Tissue Research*, 237: 31–38.

- Helbig, L., Woodbury, M.R., Haigh, J.C., Collins, J. and Barth, A.D. (2007). The seasonal fertility of North American bison (*Bison bison*) bulls. *Animal reproduction science*, 97(3-4): 265-277.
- Heuer, C., Tahir, M.N. and Amjad, H. (1987). Effect of season on fertility of frozen buffalo semen. *Animal reproduction science*, 13(1): 15-21.
- Horn, M. M., Moraes, J. C. F., & Galina, C. S. (1999). Qualidade do sêmen de touros das raças Aberdeen Angus e Brangus-Ibagéemfrente à degeneração testicular experimental induzidapordexametasona. *Ciência Rural*, 29: 523-526.
- Hultnäs, C.A. (1959). Studies on variation in mating behaviour and semen picture in young bulls of the Swedish Red and White breed and on causes of this variation. *Scandinavian Agricultural Research Workers' Association and Royal Swedish Academy of Agriculture and Forestry*.
- Ingraham, R.H., Gillette, D.D. and Wagner, W.D. (1974). Relationship of temperature and humidity to conception rate of Holstein cows in subtropical climate. *Journal of Dairy Science*, 57(4): 476-481.
- Isnaini, N., Harsi, T. and Maharani, D. (2019). Seasonal effect on semen characteristics of Murrah buffalo bulls raised under tropical climate. *Jurnal Kedokteran Hewan*, 13(3): 73-75.
- Javed, M.T., Khan, A. and Kausar, R. (2000). Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Veterinarski arhiv*, 70(2): 83-94.
- Jayendran, R. S., Vander Ven, H. H., Perez-Pelez, M., Crabo, B.G. and Zanevld, L. J. D. (1984). Development of an assay to assess the functional integrity of human sperm membrane and its relationship to other semen characters. *J. Repord. Fertil.*, 70: 219-228.
- Jiménez-Severiano, H., Quintal-Franco, J., Vega-Murillo, V., Zanella, E., Wehrman, M.E., Lindsey, B.R., Melvin, E.J. and Kinder, J.E. (2003). Season of the year influences testosterone secretion in bulls administered luteinizing hormone. *Journal of animal science*, 81(4): 1023-1029.
- Joshi, V.K. and Kharche, K.G. (1992). Assessment of sexual behavior of crossbred bulls. *Livestock Advisor*, 17(2): 38-38.

Bibliography

- Kastelic J.P., Cook, R. B., and Coulter, G. H. (1996). Contribution of the scrotum and testes to scrotal and testicular thermoregulation in bulls and rams. *J. Reprod. Fertil.*, 108: 81–5.
- Kastelic, J. P., Cook, R. B., and Coulter, G. H. (1997). Scrotal/testicular thermoregulation and the effects of increased testicular temperature in the bull. *Veterinary Clinics of North America: Food Animal Practice*, 13(2): 271-282.
- Kastelic, J.P. (2014). Understanding and evaluating bovine testes. *Theriogenology*, 81(1): 18-23.
- Kastelic, J.P., Cook, R.B. and Coulter, G.H. (1997) Contribution of the scrotum, testes, and testicular artery to scrotal/testicular thermoregulation in bulls at two ambient temperatures. *Animal reproduction science*, 45(4): 255-261.
- Kastelic, J.P., Cook, R.B., Coulter, G.H. and Saacke, R.G. (1996). Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology*, 45(5): 935-942.
- Khawaskar, M.V., Panchal, M.T., Dhami, A.J. and Patel, J.A. (2012). Seasonal variation in physico-morphological and functional characteristics of semen of Surti buffalo bulls and their interrelationships. *Indian Journal of Field Veterinarians (The)*, 8(2): 55-61.
- Klemcke, H.G., bartke, A., borer, K.T. and Hogan, M.P. (1984). Regulation of testicular prolactin and luteinizing hormone receptors in golden hamsters. *Endocrinology*, 114(2): 594-603.
- Kondracki, S., Iwanina, M., Wysokinska, A. and Gorski, K. (2013). The use of sexual activity measurements to assess ejaculatory performance of boars. *Arch. Tierzucht*. 56: 1–13
- Koonjaenak, S., Chanatinart, V., Aiumlamai, S., Pinyopumimintr, T. and Rodriguez- Martinez, H. (2007). Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. *Asian journal of andrology*, 9(1): 92-101.
- Koonjaenak, S., Pongpeng, P., Wirojwuthikul, S., Johannisson, A., Kunavongkrit, A. and Rodriguez-Martinez, H. (2007). Seasonality affects post-thaw plasma

- membrane intactness and sperm velocities in spermatozoa from Thai AI swamp buffaloes (*Bubalus bubalis*). *Theriogenology*, 67(9): 1424-1435.
- Kowalczyk, A., Gałęska, E., Czerniawska-Piątkowska, E., Szul, A. and Hebda, L., (2021). The impact of regular sperm donation on bulls' seminal plasma hormonal profile and phantom response. *Scientific Reports*, 11(1): 1-12.
- Krishna A, Singh K. (1998). Changes in the thyroid gland during the reproductive cycle of the male vespertilionid bat, *Scotophilus heathi*. *Rev Brasil Biol*, 58(4): 707-716.
- Kumar, A. and Sharma K.C. (2018). Seasonal Variation in Semen Production of Bhadawari Buffalo Bulls. *Env.Pharmacol. Life Sci.*, 7(10): 42-46
- Kumar, J, Kumar, M., Madan, A.K., Singh, Y., Yadav, B. and Anand, M. (2017). Effect of Season on Physiological Parameters and Production Profile of Haryana and Sahiwal Cattle. *J. Haryana Vet*, 56: 69-71.
- Kumar, P., Sunitha, R., Karthikeyan, A., Kumar, P. and Honnappa, T.G. (2020). Studies on effect of season on semen characteristics of Hallikar bulls. *Journal of Entomology and Zoology Studies*, 8(1): 173-176.
- Kushwaha, M.K., Bhakat, M., Mohanty, T.K., Kumar, R., Sinha, R., Rahim, A., Singh, P., Khosla, K., Shah, N. and Danish, Z. (2018). Use of scrotal infrared thermography and ultrasonography to understand thermoregulation of testis and sperm quality in Karan Fries breeding bull. *Indian Journal of Animal Research*, 53(11): 1420-1424.
- Lakhani, P., Alhussien, M.N., Lakhani, N., Jindal, R. and Nayyar, S. (2018). Seasonal variation in physiological responses, stress and metabolic-related hormones, and oxidative status of Murrah buffaloes. *Biological Rhythm Research*, 49(6): 844-852.
- Levis, D. G. and Reicks, D. L. (2005). Assessment of sexual behavior and effect of semen collection pen design and sexual stimulation of boars on behavior and sperm output-a review. *Theriogenology* 63: 630–642.
- Lincoln, G.A., Clarke, I.J. and Sweeney, T. (1996). 'Hamster- like' Cycles in Testicular Size in the Absence of Gonadotrophin Secretion in HPD Rams Exposed to

Bibliography

- Long- term Changes in Photoperiod and Treatment with Melatonin. *Journal of neuroendocrinology*, 8(11): 855-866.
- Magdub, A., Johnson, H.D. and Belyea, R.L. (1982). Effect of environmental heat and dietary fiber on thyroid physiology of lactating cows. *Journal of Dairy Science*, 65(12):2323-2331.
- Mahmood, S.A., Ijaz, A., Ahmad, N., Rehman, H.U., Zaneb, H. and Farooq, U. (2013). Studies on libido and serum testosterone concentration of Cholistani AI bulls under stress free and stressful seasons. *J Anim Plant Sci*, 23:1491-1495.
- Malama, E., Zeron, Y., Janett, F., Siuda, M., Roth, Z. and Bollwein, H. (2017). Use of computer-assisted sperm analysis and flow cytometry to detect seasonal variations of bovine semen quality. *Theriogenology*, 87:79-90.
- Mallick, S., Aggarwal, A. and Prakash, B.S. (2016). Seasonal changes in semen quality and correlation with plasma hormone profiles in Karan Fries bulls. *Biological Rhythm Research*, 47(6): 967-974.
- Mandal, D. K., Nagpaul, P. K. and Gupta, A. K. (2000). Seasonal variation in seminal attributes and sexual behaviour of Murrah buffalo bulls. *Indian J. Dairy Sci.* 53: 278-283.
- Mandal, D.K., Nagpaul, P.K. and Gupta, A.K. (2003). Motion characteristics of Murrah buffalo bull spermatozoa in various seasons and its relationship with functional integrity of the plasmallema. *Theriogenology*, 60(2): 349-358.
- Maqsood, M. (1952). Thyroid functions in relation of reproduction of mammals and birds. *Biol. Rev.* 27: 281-319.
- Marai, I. F. El-Darawany, A. A., Fadiel, A., and Abdel-Hafez, M. A. M. (2007). Physiological traits as affected by heat stress in sheep—a review. *Small ruminant research*, 71(1-3): 1-12.
- Marai, I. F. M. and Haezeb, A. A. M. (2010). Buffalo's biological functions as affected by heat stress—A review. *Livestock Science*, 127(2-3), 89-109.
- Marai, I.F., El-Darawany, A.H.A., Ismail, E.S.A.F. and Abdel-Hafez, M.A. (2006). Tunica dartos index as a parameter for measurement of adaptability of rams to subtropical conditions of Egypt. *Animal Science Journal*, 77(5): 487-494.

- Marai, I.F.M., Daader, A.H., Soliman, A.M., El-Menshawy, S.M.S. (2009). Non genetic factors affecting growth and reproduction traits of buffaloes under dry management housing (in sub-tropical environment) in Egypt. *Livest. Res. Rural. Dev.*, 21(3): 1–13.
- Marai, I.F.M., Habeeb, A.A.M. and Gad, A.E. (2002). Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livestock production science*, 78(2): 71-90.
- Mayahi, S., Mamouei, M., Tabatabaei, S. and Mirzadeh, K. (2014). Reproductive characteristics and thyroidal function in relation with season in Khuzestan buffalo (*Bubalus bubalis*) bulls. In *Veterinary research forum: an international quarterly journal*. 5(3): 201.
- McDowell, R.E., Hooven, N.W. and Camoens, J.K. (1976). Effect of climate on performance of Holsteins in first lactation. *Journal of Dairy Science*, 59(5): 965-971.
- McGreevy, P., Warren-Smith, A. and Guisard, Y. (2012). The effect of double bridles and jaw-clamping crank nosebands on temperature of eyes and facial skin of horses. *Journal of veterinary behavior*, 7(3): 142-148.
- Menegassi, S.R.O., Barcellos, J.O.J., Dias, E.A., Koetz, C., Pereira, G.R., Peripolli, V., McManus, C., Canozzi, M.E.A. and Lopes, F.G. (2015). Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. *International journal of biometeorology*, 59(3): 357-364.
- Menegassi, S.R.O., Barcellos, J.O.J., Peripolli, V. and Camargo, C.M. (2011). Behavioral assessment during breeding soundness evaluation of beef bulls in Rio Grande do Sul. *Anim. Reprod*, 8(3/4):77-80.10.
- Menegassi, S.R.O., Pereira, G.R., Dias, E.A., Rocha, M.K., Carvalho, H.R., Koetz Jr, C., Oberst, E.R. and Barcellos, J.O.J. (2018). Infrared thermography as a noninvasive method to assess scrotal insulation on sperm production in beef bulls. *Andrologia*, 50(3): 12904.
- Mengwei, L., Hassan, F.U., Yanxia, G., Zhenhua, T., Xin, L., Fang, X., Lijuan, P. and Yang, C., (2021). Seasonal dynamics of physiological, oxidative and

Bibliography

- metabolic responses in non-lactating Nili-Ravi buffaloes under hot and humid climate. *Frontiers in Veterinary Science*, 7: 622.
- Meyerhoeffer, D. C., Wettemann, R. P., Coleman, S. W. and Wells, M. E. (1985). Reproductive criteria of beef bulls during and after exposure to increased ambient temperature. *Journal of Animal Science*, 60(2): 352-357.
- Michael, J.D., Ghuman, S.S., Neglia, G., Della Valle, G., Baruselli, P.S., Zicarelli, L., Visintin, J.A., Sarkar, M. and Campanile, G. (2020). Exogenous and endogenous factors in seasonality of reproduction in buffalo: a review. *Theriogenology*, 150: 186-192.
- Mieusset, R. and Bujan, L. (1995). Testicular heating and its possible contributions to male infertility: a review. *International journal of andrology*, 18(4):169-184.
- Minton, J.E. (1994). Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. *Journal of animal science*, 72(7):1891-1898.
- Minton, J.E., Wettemann, R.P., Meyerhoeffer, D.C., Hintz, R.L. and Turman, E.J. (1981). Serum luteinizing hormone and testosterone in bulls during exposure to elevated ambient temperature. *Journal of Animal Science*, 53(6):1551-1558.
- Mishra, S.R. (2021). Thermoregulatory responses in buffaloes against heat stress: An updated review. *Journal of Thermal Biology*, 96: 1028-44.
- Misra, N.S. and Sen Gupta, B.P. (1965). Climatic environment and reproductive behaviour of buffalos: 3. Observations on semen quality of buffalo bulls maintained under two different housing conditions. *Indian Vet. J.* 65:61-64
- Moran, J.B. (1973). Heat tolerance of Brahman cross, buffalo, Banteng and Shorthorn steers during exposure to sun and as a result of exercise. *Australian Journal of Agricultural Research*, 24(5):775-782.
- Morrell, J.M. (2020). Heat stress and bull fertility. *Theriogenology*.153: 62-67.
- Nääs, I. A., Garcia, R. G., and Caldara, F. R. (2020). Infrared thermal image for assessing animal health and welfare. *Journal of Animal Behaviour and Biometeorology*, 2(3): 66-72.

- Nagarcenkar, R. and Sethi, R.K., (1981). Association of adaptive traits with performance traits in buffaloes. *Indian Journal of Animal Sciences (India)*.
- Naskar, S. and Nagpaul, P.K. (2005). Effect of social facilitation on seminal attributes and sexual behavior in crossbred male goats. *Indian J. Anim. Prod. Mgmt.*, 19(4):103-108.
- Nichi, M., Bols, P.E.J., Züge, R.M., Barnabe, V.H., Goovaerts, I.G.F., Barnabe, R.C. and Cortada, C.N.M. (2006). Seasonal variation in semen quality in Bosindicus and Bostaurus bulls raised under tropical conditions. *Theriogenology*, 66(4): 822-828.
- Nitharwal, K. (2013). Post thaw sperm functions of Murrah buffalo bull frozen during different season. *M.V.Sc. Thesis*. NDRI, Deemed University, Karnal, Haryana, India.
- Nitharwal, K., Singh, P., Bhakat, M., Chaurasia, A., Abdullah, M. and Prabha, C. (2017). Effect of season on semen quality parameters in Murrah buffalo. *Indian Journal of Animal Sciences*, 87(1):64-69.
- Parmar, K.H., Tank P.H. and Kavani P.H. (2019). Seasonal Influence on Post Thaw Seminal Attributes and Conception Rate from Semen of Jaffarabadi Buffalo Bulls. *Indian Journal of Animal Research*.
- Paul, C., Murray, A. A., Spears, N., & Saunders, P. T. (2008). A single, mild, transient scrotal heat stress causes DNA damage, sub fertility and impairs formation of blastocysts in mice. *Reproduction*, 136(1): 73.
- Paul, C., Teng, S., and Saunders, P. T. (2009). A single, mild, transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. *Biology of reproduction*, 80(5): 913-919.
- Payne, J.M. and Payne, S. (1987). Indicators of blood enzymes. In: The metabolic profile test. (Payne JM, Payne S, Eds.) *Oxford university press*. Oxford, New York, Tokyo, 92-100.
- Peirce, A.R.J., Downey, B.R. and Sanford, L.M. (1987). Seasonal changes in plasma concentrations of prolactin, LH, FSH, and testosterone in young adult bulls. *Animal Reproduction Science*, 13(3): 165-176.

Bibliography

- Perera, B.M.A.O., Pathiraja, N., Motha, M.X.J. and Weerasekera, D.A. (1979). Seasonal differences in plasma testosterone profiles in buffalo bulls. *Theriogenology*, 12(1): 33-38.
- Peris, S.I., Bilodeau, J.F., Dufour, M. and Bailey, J.L. (2007). Impact of cryopreservation and reactive oxygen species on DNA integrity, lipid peroxidation, and functional parameters in ram sperm. *Molecular reproduction and development*, 74(7): 878-892.
- Perry, V.E.A., Chenoweth, P.J., Post, T.B. and Munro, R.K. (1991). Patterns of development of gonads, sex-drive and hormonal responses in tropical beef bulls. *Theriogenology*, 35(2): 473-486.
- Perumal, P., Chang, S., De, A.K., Baruah, K.K., Khate, K., Vupru, K. and Mitra, A. (2020). Slow release exogenous melatonin modulates scrotal circumference and testicular parameters, libido, endocrinological profiles and antioxidant and oxidative stress profiles in mithun. *Theriogenology*. 154: 1-10
- Perumal, P., De, A.K., Alyethodi, R.R., Savino, N., Khate, K., Vupru, K. and Khan, M.H. (2021). Daily and seasonal rhythmic secretary pattern of endocrinological profiles in mithun bull. *Theriogenology*, 166: 46-54.
- Perumal, P., Savino, N., Sangma, C.T.R., Khan, M.H., Ezung, E., Chang, S. and 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.
- Pound, N., Javed, M. H., Ruberto, C., Anis, S. M., Del and Valle, A. P. (2002). Duration of sexual arousal predicts semen parameters for masturbatory ejaculates. *Physiol. Behav.* 76: 685–689.
- Purwanto, B.P., Abo, Y., Sakamoto, R., Furumoto, F. and Yamamoto, S. (1990). Diurnal patterns of heat production and heart rate under thermoneutral conditions in Holstein Friesian cows differing in milk production. *The Journal of Agricultural Science*, 114(2): 139-142.
- Rahman, M.B., Vandaele, L., Rijsselaere, T., Maes, D., Hoogewijs, M., Frijters, A., Noordman, J., Granados, A., Dernelle, E., Shamsuddin, M. and Parrish, J.J. (2011). Scrotal insulation and its relationship to abnormal morphology,

- chromatin protamination and nuclear shape of spermatozoa in Holstein-Friesian and Belgian Blue bulls. *Theriogenology*, 76(7): 1246-1257.
- Ram, K. L., Sahasrabudhe, S. A., Tiwari, R. P., Mishra, G. K., and Nair, A. K. (2017). Effect of heat stress on seminal characteristics of murrh buffalo bull semen. *Buffalo Bulletin*, 36(2): 369-378.
- Ramadan, T.A., Kumar, D., Ghuman, S.S. and Singh, I. (2019). Melatonin-improved buffalo semen quality during non breeding season under tropical condition. *Domestic animal endocrinology*, 68: 119-125.
- Ramadan, T.A., Tahab, T.A., Samakb, M.A. and Hassanb, A. (2009). Seasonal and monthly variations in semen characteristics of Egyptian buffalo bulls. *Alex. J. Agric. Res*, 54: 13-23.
- Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana, P.R. and Bhatta, R. (2020). Physiological adaptability of livestock to heat stress: an updated review. *Journal of Animal Behaviour and Biometeorology*, 6(3): 62-71.
- Rathwa, S.D., Vasava, A.A., Pathan, M.M., Madhira, S.P., Patel, Y.G. and Pande, A.M. (2017). Effect of season on physiological, biochemical, hormonal, and oxidative stress parameters of indigenous sheep. *Veterinary world*, 10(6): 650-654.
- Reece, W.O., Erickson, H.H., Goff, J.P. and Uemura, E.E. eds. (2015). *Dukes' physiology of domestic animals*. John Wiley & Sons.
- River, C. and Rivest, S. (1991). Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. *Biology of reproduction*, 45(4): 523-532.
- Rizzoto, G. and Kastelic, J.P. (2020). A new paradigm regarding testicular thermoregulation in ruminants. *Theriogenology*, 147: 166-175.
- Rizzoto, G., Ferreira J.C.P., Garcia H.D.M., Teixeira-Neto, F.J., Bardella, L.C., Martins, C.L. (2020). Short-term testicular warming under anesthesia causes similar increases in testicular blood flow in *Bos taurus* versus *Bos indicus* bulls, but no apparent hypoxia. *Theriogenology*, 145: 94-9.

Bibliography

- Sabés-Alsina, M., Lundeheim, N., Johannisson, A., López-Béjar, M. and Morrell, J.M. (2019). Relationships between climate and sperm quality in dairy bull semen: A retrospective analysis. *Journal of dairy science*, 102(6): 5623-5633.
- Sajjad, M., Ali, S., Ullah, N., Anwar, M., Akhter, S. and Andrabi, S.M.H. (2007). Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in Nili-Ravi buffalo bulls. *Pakistan Veterinary Journal*, 27(2): 63.
- Sanocka, D. and Kurpisz, M. (2004). Reactive oxygen species and sperm cells. *Reproductive Biology and Endocrinology*, 2(1):1-7.
- Santos V.P. (2003). Stress and reproduction. Seminar, Federal University of Rio Grande do Sul.
- Sayah, M.S., El-Hamd, M.A.A., El-Diahy, Y.M. and Nagadi, S.A. (2019). Effect of Season and Housing on Physiological Reactions, Testosterone and Semen Quality of Friesian Bulls. *International Journal of Engineering Research & Technology*, 8(10): 2278-0181.
- Seifi-Jamadi, A., Zhandi, M., Kohram, H., Luceño, N.L., Leemans, B., Henrotte, E., Latour, C., Demeyere, K., Meyer, E. and Van Soom, A. (2020). Influence of seasonal differences on semen quality and subsequent embryo development of Belgian Blue bulls. *Theriogenology*, 158: 8-17.
- Setchell, B. (1978). The scrotum and thermoregulation. In *The Mammalian Testis* (ed. B. Setchell), 90–108. Ithaca: Cornell University Press.
- Setchell, B. P. (1998). The parkes lecture heat and the testis. *Reproduction*, 114(2): 179-194.
- Shafie, M.M. (1985). Physiological responses and adaptation of water buffalo. *Stress physiology in livestock. Volume II. Ungulates.*, 67-80.
- Shafie, M.M. and El-Khair, M.M.A. (1970) .Activity of the sebaceous glands of bovines in hot climates. *United Arab Republic Journal of Animal Production*, 10(1): 81-98.
- Sharma, M., Bhat, Y., Sharma, N. and Singh, A. (2018). Comparative study of seasonal variation in semen characteristics of buffalo bull. *Journal of Entomology and Zoology Studies*, 6(1): 52-109.

- Sharma, M., Yaqoob, B., Singh, A., Sharma, N. and Rawat, S. (2017). Effect of temperature humidity index on semen quality of bovine bull. *International Journal of Current Microbiology and Applied Sciences*, 6(12): 1822-1830.
- Sholikah, N., Supriyanto, S., Yekti, A., Kuswati, S. and Susilawati, T. (2018). Sexual behaviour and semen production of Madura bulls. *Intl. Res. J. Adva. Engg. and Sci.*, 4(1): 48-50
- Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock production science*, 67(1-2): 1-18.
- Silva, J., Araújo, A., Lourenço Júnior, J., Santos, N., Viana, R.B., Garcia, A.R., Rondina, D. and Grise, M.M. (2014). Hormonal changes in female buffaloes under shading in tropical climate of Eastern Amazon, Brazil. *Revista Brasileira de Zootecnia*, 43: 44-48.
- Singh, A.K., Devi, R., Kumar, Y., Kumar, P., and Upadhyay, R.C. (2014). Physiological changes and blood flow in Murrah buffaloes during summer and winter season. *Journal of Buffalo Science*, 3(2): 63-69.
- Singh, K., Kumar, A., Honparkhe, M. and Singh, A. K. (2019). Quantification of sexual behaviour traits in presence of teaser male in relation to Libido and semen quality in buffalo bulls. *J. Anim. Res.* 9(1): 143–146.
- Singh, V., Yadava, C., Patel, A. and kumar, A. (2018). Abnormalities in Haryana Bull Spermatozoa in Relation to Seasonal Variation. *Trends in Biosciences*, 11(21): 1-3
- Sinha, R. (2018). Digital infrared thermography as a non-invasive tool to evaluate the effect of season and vaccination stress on in-vitro fertility of Murrah bulls. *Ph. D Thesis*. NDRI, Deemed University, Karnal, Haryana, India
- Skinner, J.D. and Louw, G.N. (1966). Heat stress and spermatogenesis in *Bos indicus* and *Bos aurus* cattle. *Journal of Applied Physiology*, 21(6): 1784-1790.
- Snoj, T., Kobal, S. and Majdic, G. (2013). Effects of season, age, and breed on semen characteristics in different *Bos taurus* breeds in a 31-year retrospective study. *Theriogenology*, 79(5): 847-852.

Bibliography

- Solanki, G.B., Kavani, F.S., Talekar, S.H., Ahlawat, A.R., Savsani, H.H. and Gamit, P.M. (2019). Seasonal influence on sexual behaviour score of Gir bulls. *Int. J. Curr. Microbiol. App. Sci*, 8(6): 3100-3111.
- Soren, S., Singh, S.V. and Kumar, A. (2016). Influence of season on semen quality in Karan Fries (Tharparkar× Holstein Friesian) bulls. *Journal of Animal Research*, 6(2): 301-305.
- Srikandakumar, A. and Johnson, E.H. (2004). Effect of heat stress on milk production, rectal temperature, respiratory rate and blood chemistry in Holstein, Jersey and Australian Milking Zebu cows. *Tropical Animal Health and Production*, 36(7): 685-692.
- Stålhammar, E.M., Janson, L. and Philipsson, J. (1989). Genetic studies on fertility in AI bulls. I. Age, season and genetic effects on semen characteristics in young bulls. *Animal Reproduction Science*, 19(1-2): 1-17.
- Stewart, M., Webster, J.R., Verkerk, G.A., Schaefer, A.L., Colyn, J.J. and Stafford, K.J. (2007). Non-invasive measurement of stress in dairy cows using infrared thermography. *Physiology & behavior*, 92(3): 520-525.
- Stumpf, T.T., Wolfe, M.W., Roberson, M.S., Kittok, R.J. and Kinder, J.E. (1993). Season of the year influences concentration and pattern of gonadotropins and testosterone in circulation of the bovine male. *Biology of reproduction*, 49(5): 1089-1095.
- Takahasi, K., Vehida, A. and Kitao, M. (1990). Hypo- Osmotic test of sperm. *Arch. Androl.*, 25: 225 – 242.
- Tatman, S.R., Neuendorff, D.A., Wilson, T.W. and Randel, R.D. (2004). Influence of season of birth on growth and reproductive development of Brahman bulls. *Theriogenology*, 62(1-2): 93-102.
- Taylor, N.A., Tipton, M.J. and Kenny, G.P. (2014). Considerations for the measurement of core, skin and mean body temperatures. *Journal of thermal biology*, 46: 72-101.
- Thrun, L.A., Dahl, G.E., Evans, N.P. and Karsch, F.J. (1997). A critical period for thyroid hormone action on seasonal changes in reproductive neuroendocrine function in the ewe. *Endocrinology*, 138(8): 3402-3409.

- Titto, C. G., Titto, E. A. L., Titto, R. M. and Mourao, G. B. (2011). Heat tolerance and the effects of shade on the behavior of Simmental bulls on pasture. *Animal Science Journal*, 82(4): 591-600.
- Tiwari, R., Mishra, G.K., Shukla, M.K., Singh, R.B., Saxena, S.K. and Siddiqui, M.U. (2011). Seasonal variation in semen production of Murrah buffalo bulls. *Indian J. Anim. Reprod*, 32(2):52-54.
- Tomar, N.S., Mishra, B.S. and Johari, C.B. (1966). Seasonal variations in reaction time and semen production, and prediction of some semen attributes on initial motility of spermatozoa in Haryana and Murrah bulls. *Indian J. Dairy Sci*, 19(1): 87-93.
- Tucker, H.A., Koprowski, J.A., Britt, J.H. and Oxender, W.D. (1974). Serum prolactin and growth hormone in Holstein bulls. *Journal of dairy science*, 57(9): 1092-1094.
- Tuli, R.K. and Singh, M. (1983). Seasonal variation in freezability of buffalo semen. *Theriogenology*, 20(3): 321-324.
- Uddin, J., McNeill, D.M., Lisle, A.T. and Phillips, C.J. (2020). A sampling strategy for the determination of infrared temperature of relevant external body surfaces of dairy cows. *International Journal of Biometeorology*, 64: 1583-1592.
- Uddin, J., Phillips, C.J., Goma, A.A. and McNeill, D.M. (2019). Relationships between infrared temperature and laterality. *Applied Animal Behaviour Science*, 220: 1048-55.
- Valeanu, S., Johannisson, A., Lundeheim, N. and Morrell, J.M. (2014). Seasonal variation in sperm quality parameters in Swedish red dairy bulls used for artificial insemination. *Livestock Science*, 173: 111-118.
- Veissier, I., Palme, R., Moons, C.P., Ampe, B., Sonck, B., Andanson, S. and Tuytens, F.A. (2018). Heat stress in cows at pasture and benefit of shade in a temperate climate region. *International journal of biometeorology*, 62(4): 585-595.
- Vermunt, J. J. and Tranter, B. P. (2011). Heat stress in dairy cattle—a review, and some of the potential risks associated with the nutritional management of this condition. In *review of AVA QLD Division Conference 25-27/3/10 Australian Veterinary Association*, 212-221.

Bibliography

- Waites, G. M. H. (1970). Temperature regulation and the testis. *Development, anatomy, and physiology*, 241-279.
- Wankhade, P.R., Diwakar, V.K., Talokar, A.J., Aderao, G.N., Miranda, C.D. and Gourkhede, D.P. (2015). Effect of photoperiod on the performances of Buffaloes: A review. *Journal of Entomology and Zoology Studies*, 7(1): 177-180.
- Warwick, E.C., Childa, C.E., Flower, A.E. and Ham, W. E. (1948). Ram semen production and characteristics as influenced by administration of thyroprotein. *J. Anim. Sci.*, 7: 198-207.
- Watson P. F. (1975). Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Vet Rec.*, 97(1): 12-5.
- West, J.W. (1994). Interactions of energy and bovine somatotropin with heat stress. *Journal of Dairy Science*, 77(7): 2091-2102.
- Whittow, G.C. (1965). The effect of hyperthermia on the systemic and pulmonary circulation of the ox (*Bostaurus*). *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences: Translation and Integration*, 50(3): 300-311.
- Yadav, S.K., Singh, P., Kumar, P., Bhakat, M., Singh, A., Mondal, S. and Patel, B. (2017). Relationship among testicular, physical and semen quality parameters of Murrah buffalo breeding bulls. *Indian J Dairy Sci*, 70(4): 462-465.