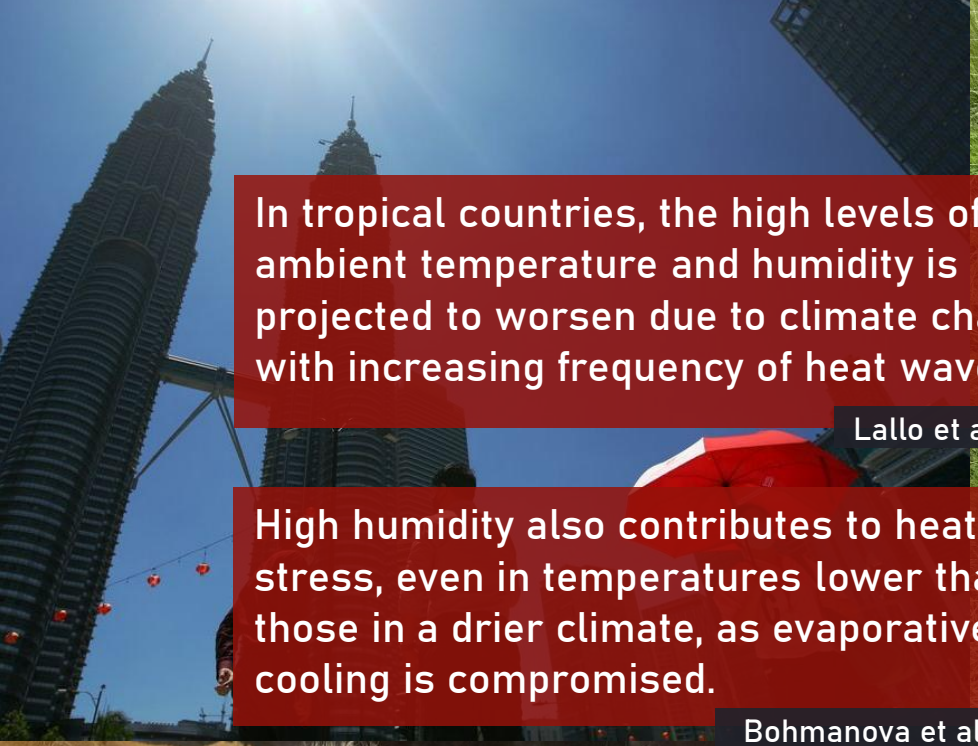
Impact of heat stress on reproductive performance in Barbados Blackbelly rams

Dr. Nuradilla Mohamad Fauzi
Institute of Biological Sciences, Faculty of Science
Glami Lemi Biotechnology Research Centre
Universiti Malaya, Kuala Lumpur, Malaysia

The 4th seminar on "Biotechnology in small ruminant reproduction: an international experience – 2025" ČZU Prague| 5th May 2025

UNIVERSITI MALAYA

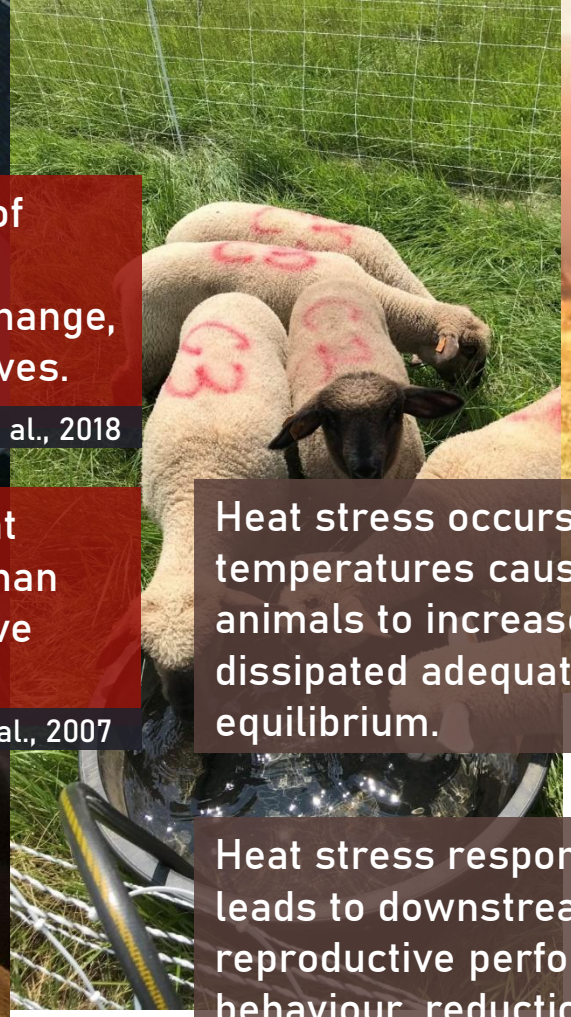


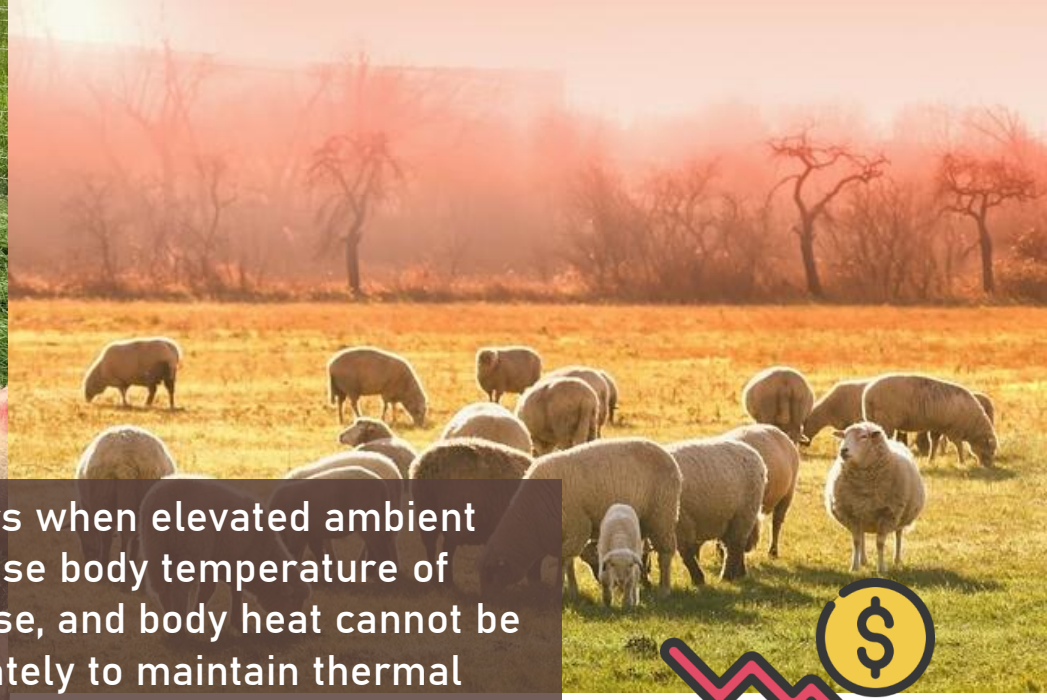
In tropical countries, the high levels of ambient temperature and humidity is projected to worsen due to climate change, with increasing frequency of heat waves.

Lallo et al., 2018

High humidity also contributes to heat stress, even in temperatures lower than those in a drier climate, as evaporative cooling is compromised.

Bohmanova et al., 2007





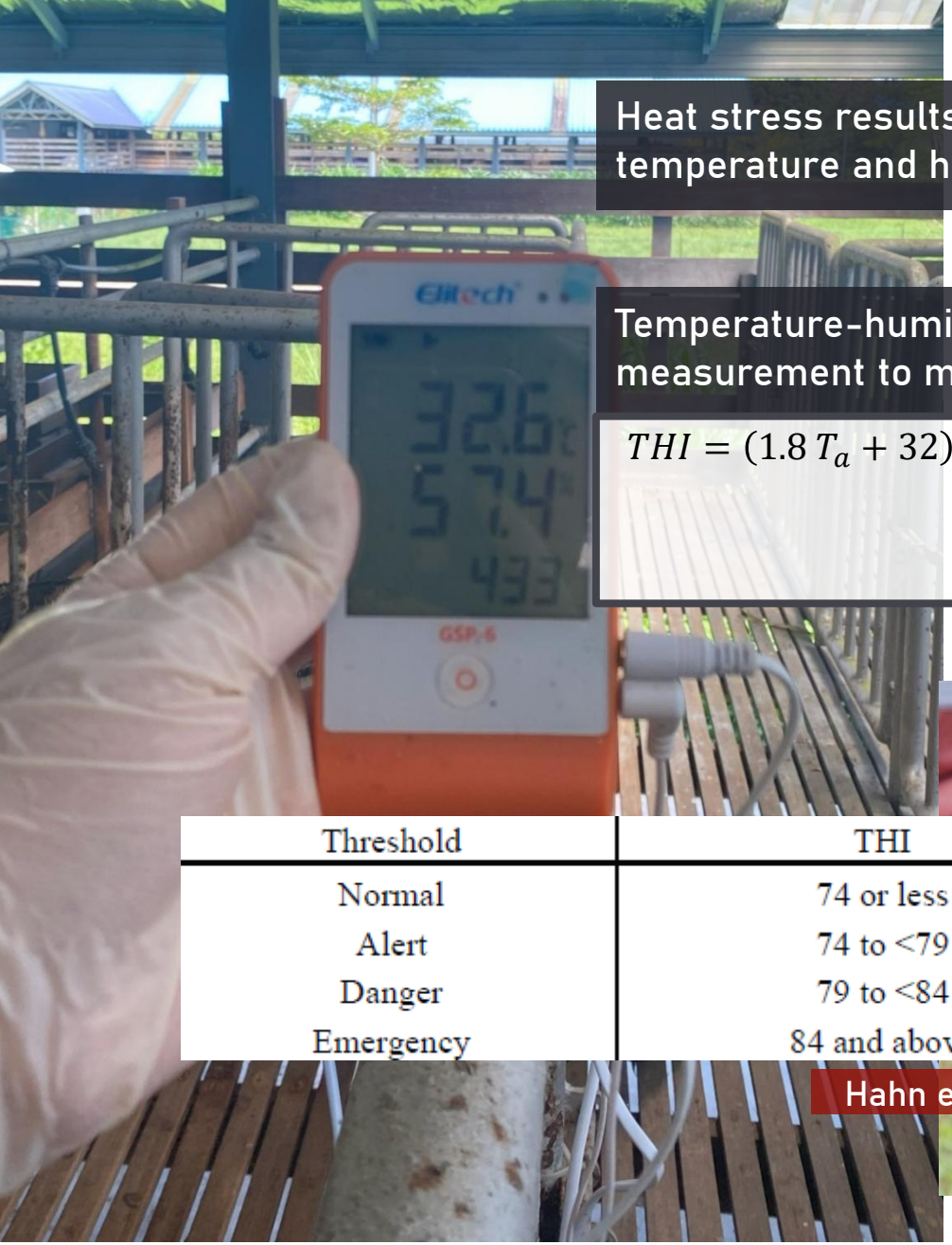
Heat stress occurs when elevated ambient temperatures cause body temperature of animals to increase, and body heat cannot be dissipated adequately to maintain thermal equilibrium.

Ji et al., 2020; Wang et al., 2020

Heat stress response in animals leads to downstream effects in reproductive performance, behaviour, reduction in reproductive output, feed intake and body weight gain.

Sejian et al., 2014; 2019

Risk to production of livestock products and food/economic security.



Heat stress results from the interaction between temperature and humidity.

Wijffels et al., 2018

Temperature-humidity index (THI) is an established measurement to monitor heat stress in livestock.

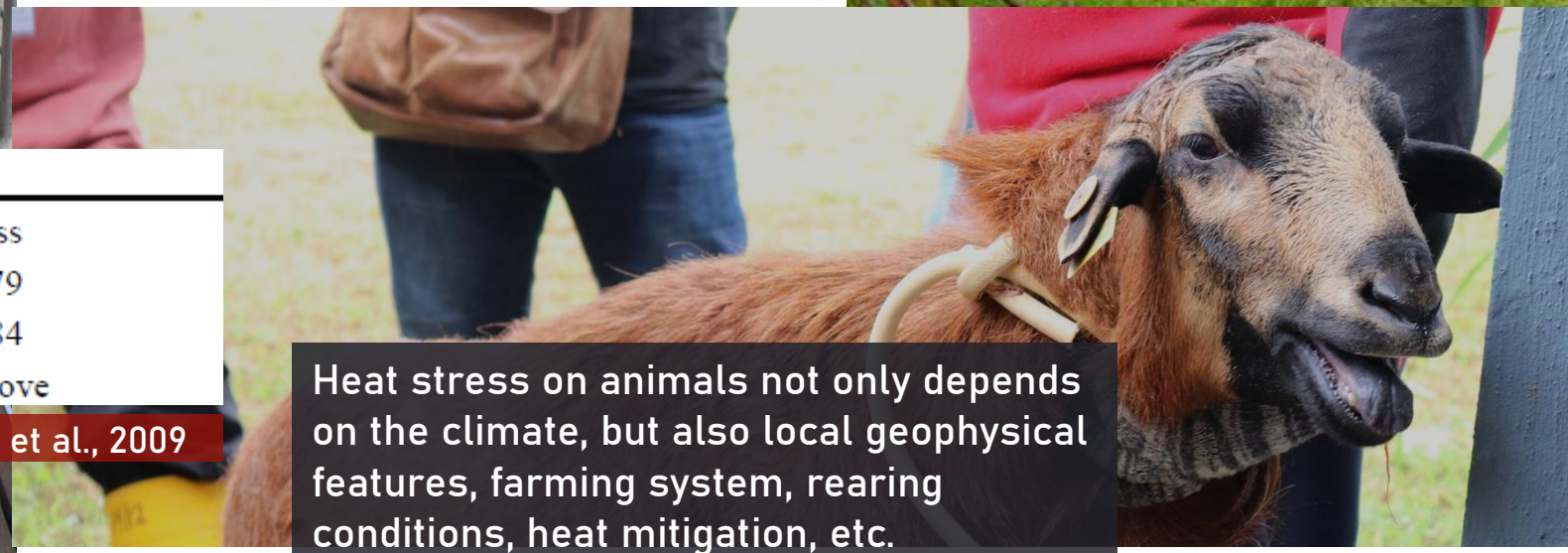
$$THI = (1.8\,T_a + 32) - ((0.55 - 0.0055\mathrm{RH})(1.8T_a - 26.8))$$

T_a = ambient temperature (°C);
RH = relative humidity

Lallo et al., 2018

Threshold	THI
Normal	74 or less
Alert	74 to <79
Danger	79 to <84
Emergency	84 and above

Hahn et al., 2009



Heat stress on animals not only depends on the climate, but also local geophysical features, farming system, rearing conditions, heat mitigation, etc.

Bohmanova et al., 2007; Lopes et al., 2016; Lallo et al., 2018; Madhusoodan et al., 2020

High dependency on mutton/lamb imports.
Sheep farming in Malaysia is dominated by smallholder farmers.

Department of Statistics Malaysia, 2020

THI monitoring for livestock is widespread around the world for sustainability and welfare, but lacking in Malaysia.

Polsky & von Keyserlingk, 2017; Islam et al., 2021

Small ruminants reared in tropical regions are at constant risk of heat stress, due to the consistently hot and humid environment.

McManus et al., 2022

Important to monitor and manage heat stress efficiently!

BARBADOS BLACKBELLY (BB) SHEEP

First imported into Malaysia in 1993

Malaysian Department of Veterinary Services., 2013

Prolific sheep breed characterised by their tropical adaptability, high proliferation rate, lower lamb mortality rate and tolerance to diseases and parasites.

Lallo et al., 2018; de Almeida et al., 2018

Hair-type breeds like BB are more suitable for mutton production in Malaysia due to their tropical adaptability, with high potential to improve local sheep production.

Malaysian Department of Veterinary Services., 2013

The downstream effects of heat stress on reproductive performance have never been characterised for Barbados Blackbelly in the context of Malaysian tropical climate.

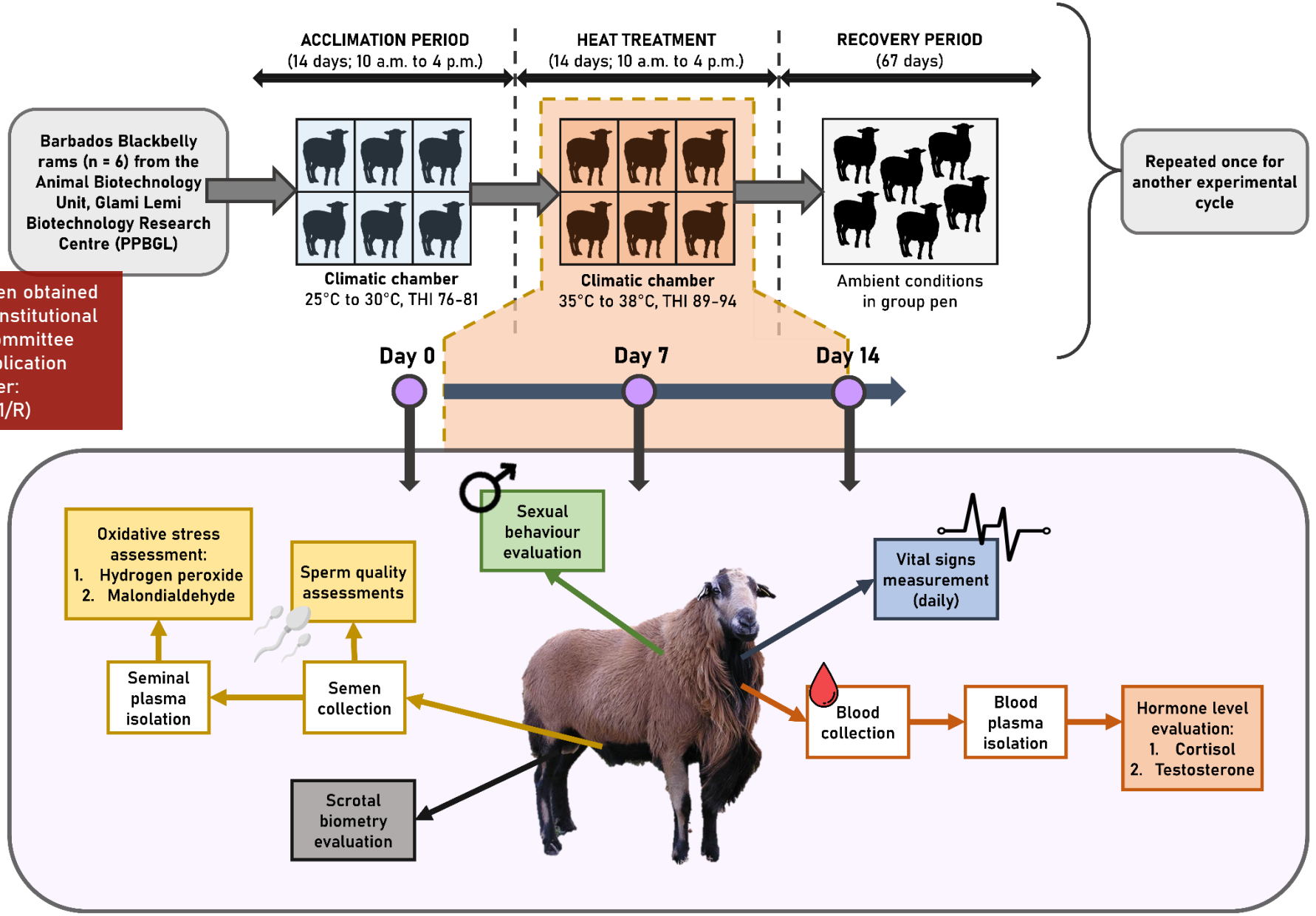
Does heat stress induced by high THI affect reproductive performance in Barbados Blackbelly rams?

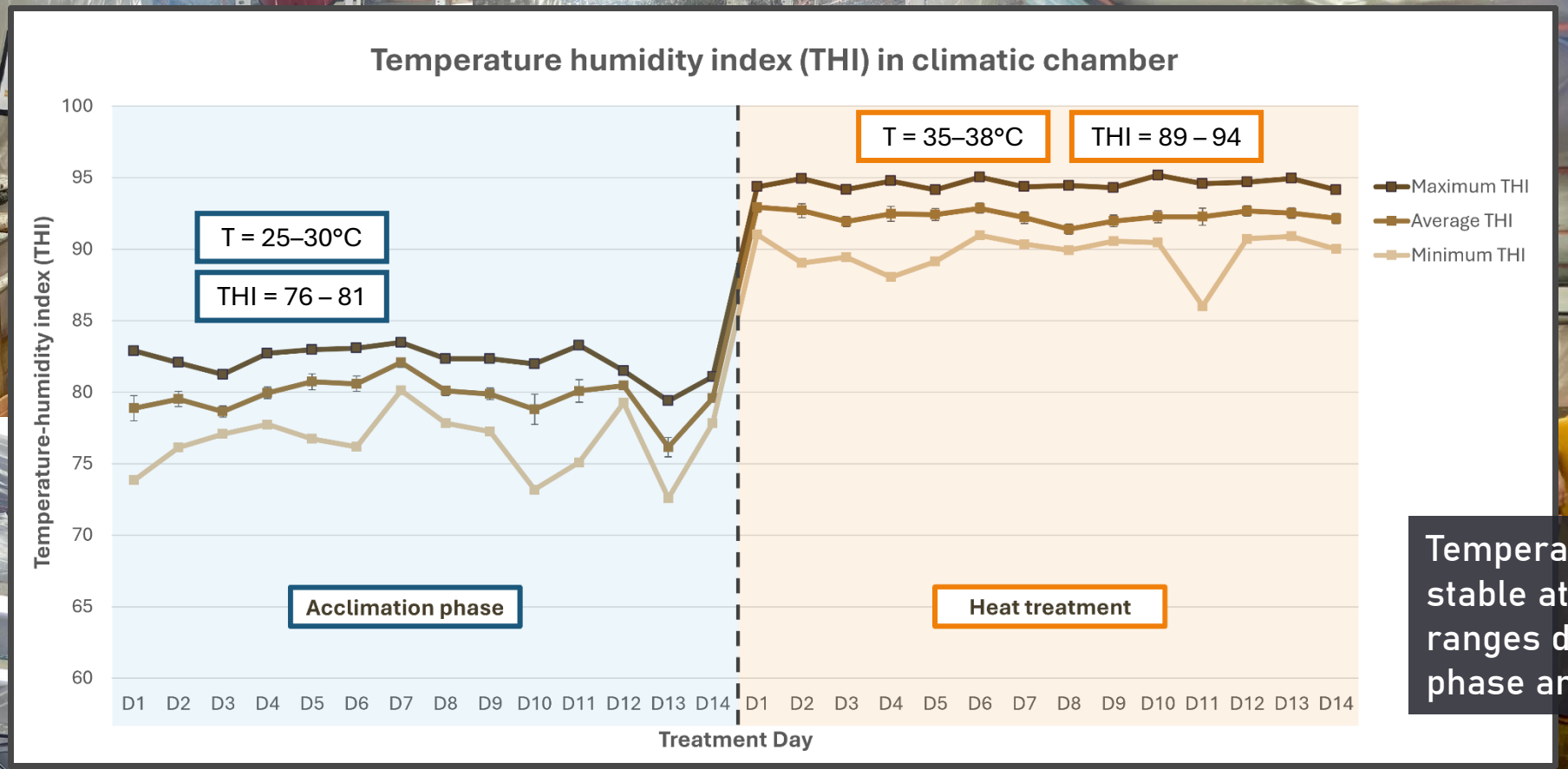
How does heat stress affect sperm quality and sexual behavior?

Are Barbados Blackbelly rams tolerant towards heat stress in Malaysian tropical climate?

Does heat stress induce oxidative stress?

Ethical approval has been obtained from Universiti Malaya Institutional Animal Care and Use Committee through Animal Use Application (Ethics reference number: S/26062023/17052023-01/R)





Temperature and THI were stable at the desired ranges during acclimation phase and heat treatment!

Exhaust fan

VITAL SIGN PARAMETERS

- Elevation in rectal temperature, heart rate and respiration rate resulted from the employment of physiological thermoregulation mechanisms to dissipate the excess heat load when subjected to a high THI environment.

Berihulay et al., 2019

- Elevation in testicular temperature could result in impaired sperm quality, when the temperature rises above the ideal range for spermatogenesis.

Robinson et al., 2023

Roslan et al. Reproductive physiological and behavioural adaptability of Barbados Blackbelly rams under elevated temperature-humidity index. (manuscript in prep)

- Cortisol level was highest in the rams at Day 7 of heat treatment.

- Cortisol is a glucocorticoid hormone responsible for stress response in animals. Elevated ambient temperatures increased cortisol production to relieve heat stress.

Hahn et al., 2009; Wijffels et al., 2018

- The decrease in cortisol concentration at Day 14 might indicate physiological adaptation to heat stress at that point of the treatment.

Roslan et al. Reproductive physiological and behavioural adaptability of Barbados Blackbelly rams under elevated temperature-humidity index. (manuscript in prep)

- The increased production of cortisol to relieve heat stress inevitably lowers the secretion of gonadotrophin releasing hormone (GnRH), which affects the release of hormones vital for development and reproduction.

Das et al., 2016; De et al., 2017

- Lowered GnRH levels ultimately leads to decreased production of testosterone.

Dobson et al., 2012

Roslan et al. Reproductive physiological and behavioural adaptability of Barbados Blackbelly rams under elevated temperature-humidity index. (manuscript in prep)

SEXUAL BEHAVIOUR

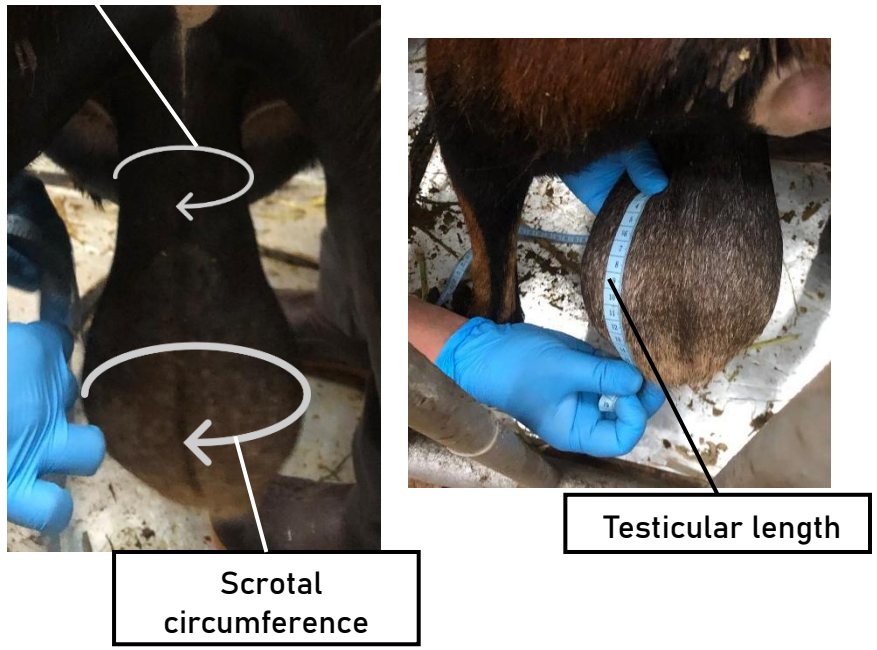
- Heat stress caused lowered testosterone levels, which constituted to decreased libido (courting desire). This led to increased ejaculation time and number of mountings until ejaculation.

Maurya et al., 2018

- Lowered libido could also cause increased number of kicking and sniffing – due to increase in unsuccessful mountings and ejaculation in the rams.

Roslan et al. Reproductive physiological and behavioural adaptability of Barbados Blackbelly rams under elevated temperature-humidity index. (manuscript in prep)

SCROTAL BIOMETRY



Reduction in scrotal and testicular measurements could be attributed to:

- Histopathological shrinkage of testicular tissues and seminiferous tubules.
- Degeneration of Sertoli and Leydig cells due to increased ROS production in the testes.

Abdel-Hafez et al., 2008; Mohamed et al., 2023

- This deterioration of reproductive cells and tissues in the testes inadvertently leads to decreased sperm quality and output.

van Wettere et al., 2021

Roslan et al. Reproductive physiological and behavioural adaptability of Barbados Blackbelly rams under elevated temperature-humidity index. (manuscript in prep)

SPERM QUALITY

The overall decline in sperm quality is likely due to increased oxidative stress:

- Reactive oxygen species (ROS) accumulation increases lipid peroxidation in sperm cells, leading to their decreased motility and morphological alterations.
- Increased ROS level damages testicular tissues and Leydig and Sertoli cells, causing the deterioration of the developing germ cells.

Capela et al., 2022; Ben Moula et al., 2024

OXIDATIVE STRESS IN SEMINAL PLASMA

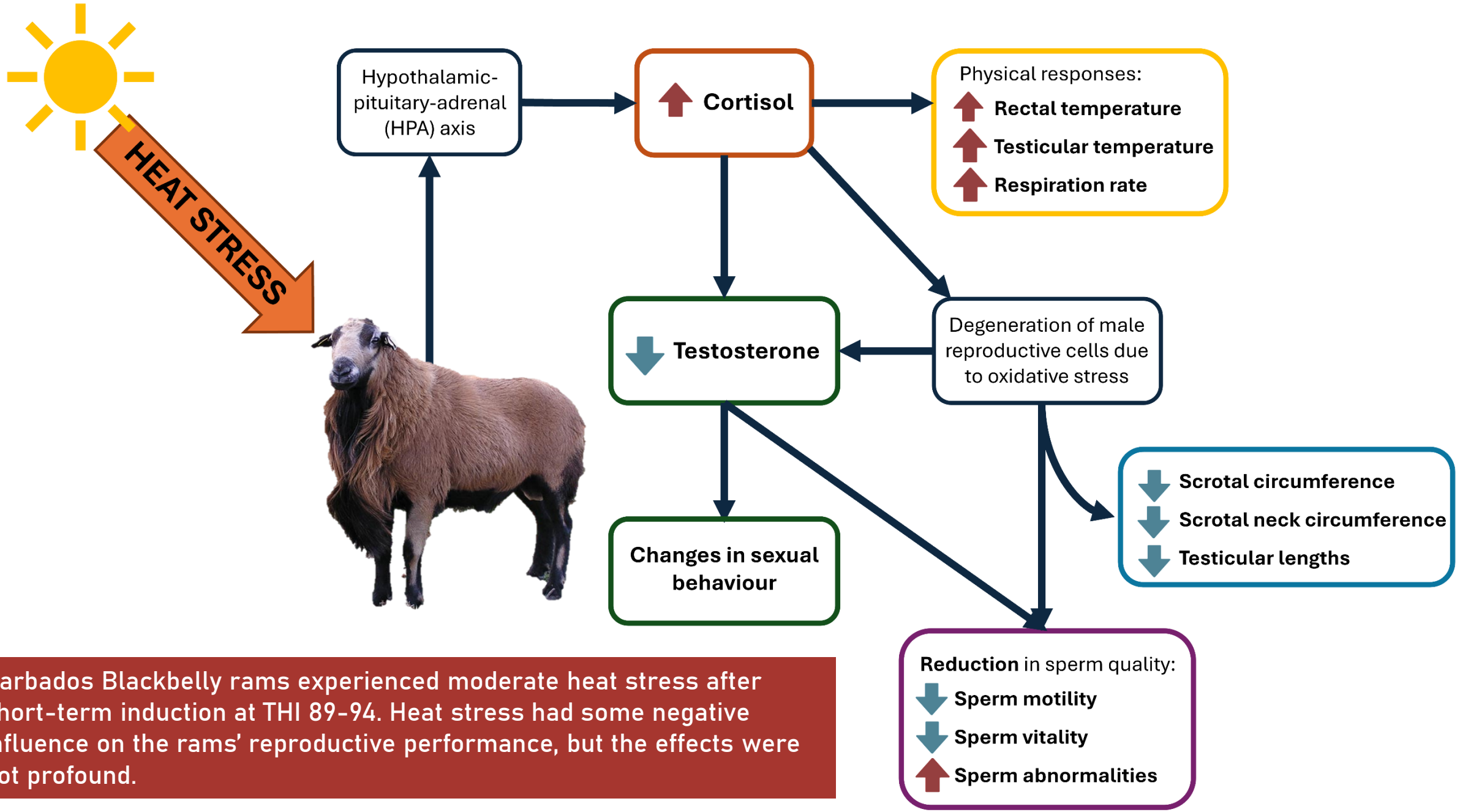
- In response to heat stress, increased secretion of cortisol activates the glucocorticoid receptors in animal cells, which triggers various reactions producing ROS and free radicals such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2·—) and hydroxyl radicals (·OH).

Spiers et al., 2015

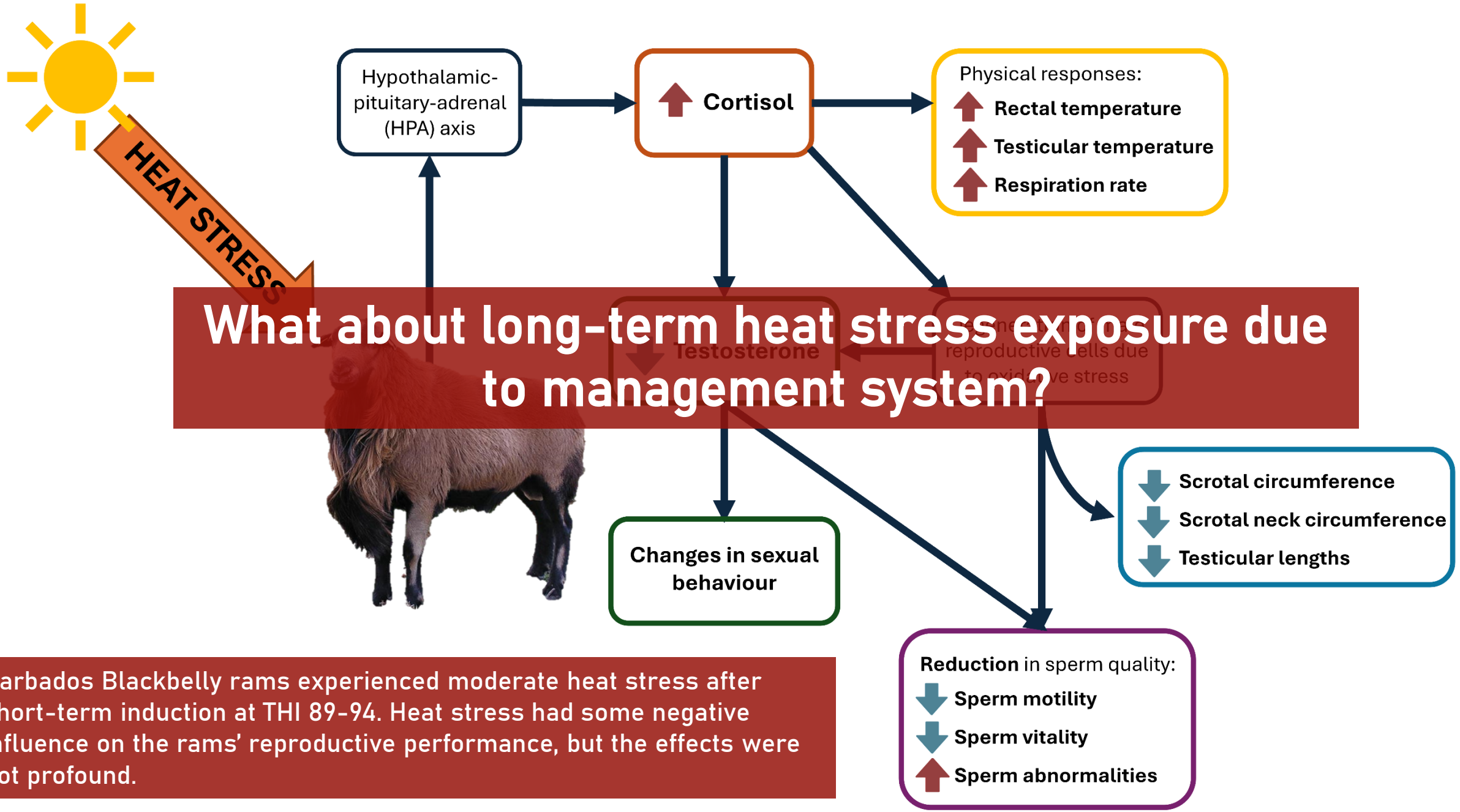
- Despite being insignificant, the increasing trend of H_2O_2 and malondialdehyde (MDA) concentration in BB ram seminal plasma might indicate increased oxidative stress status, leading to the decline in sperm quality.

McManus et al., 2020

Roslan et al. Reproductive physiological and behavioural adaptability of Barbados Blackbelly rams under elevated temperature-humidity index. (manuscript in prep)



Barbados Blackbelly rams experienced moderate heat stress after short-term induction at THI 89-94. Heat stress had some negative influence on the rams' reproductive performance, but the effects were not profound.



Barbados Blackbelly rams experienced moderate heat stress after short-term induction at THI 89-94. Heat stress had some negative influence on the rams' reproductive performance, but the effects were not profound.

Common management systems in Southeast Asia includes intensive, semi-intensive and extensive/free range.

Intensive farming is higher in cost.

Siasiou et al., 2021

Semi-intensive/extensive livestock are more exposed to the negative impact of climate change compared to intensive system.

Nardone et al., 2010

Semi-intensive livestock tends to have higher body temperature than intensively reared animals.

Adama et al., 2018

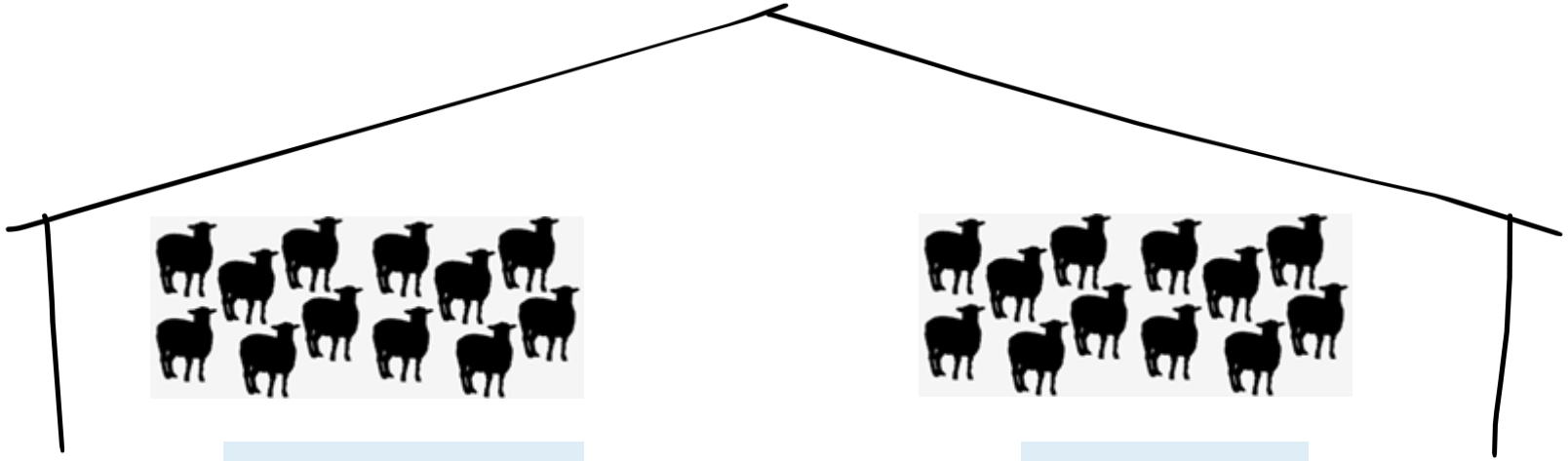
Currently, there is a lack of research in Malaysia and Southeast Asian countries on heat stress effect by management systems, especially on small ruminant reproductive performance.

Do different management systems impact the occurrence of heat stress in Barbados Blackbelly rams?

Are rams in intensive or semi-intensive systems more exposed to high THI?

Intensive (ITS)

Semi-Intensive (SIS)



n = 12
24h indoors

n = 12

6 hours outdoors
(no shade)
9 am – 3 pm

Barbados Blackbelly rams
(2-3 yo, 48-60 kg BW)
Ad-libitum water + fixed diet regime
8 weeks x 3 cycles

PHYSIOLOGICAL RESPONSES

- ITS **RR showed significant correlation with THI** – RR is more sensitive and effective mechanism in removing heat load excess (Silva et al., 2024).
  - ITS physiological responses were not significantly affected by THI.
  - Pearson correlation indicated that the ITS physiological responses (RT, TT, HR) were not significantly correlated with THI.
- **SIS rams significantly affected**; HR, RT and RR are good indicators for heat stress occurrence in small ruminants (Sejian et al., 2017; Al-Dawood, 2007; Marai et al., 2007);
- **SIS rams significantly exposed to HS** than ITS. All physiological parameters correlated with THI

PHYSIOLOGICAL RESPONSES

- **Solar radiation (in SIS)** induce physiological responses for thermoregulation and energy metabolism (van Wettere et al., 2021; Schütz et al., 2024)
- **Severity of THI does not reflect the heat stress effect in ITS rams based on physiological responses (Table 3):** Barbados Blackbelly adaptable traits towards tropics climate (Horton & Burgher, 1992 ; de Almeida et al., 208; Paim et al., 2021)
  … or the **established THI ranges are not suitable for tropical countries?**

Mazlishah et al. Influence of the Temperature-Humidity Index (THI) on the Sperm Quality of Barbados Blackbelly Rams under Different Management Systems in the Tropics (manuscript in prep)

REPRODUCTIVE PERFORMANCE

- Study by Maurya et al. (2017) reported significantly longer time for first ejaculation and no significant difference for number of mounting after exposed to 42 ºC for 2 months.

- Implying that the severity of HS exposed to rams (SIS) in current study does not impact reproductive behaviour severely.

Mazlishah et al. Influence of the Temperature-Humidity Index (THI) on the Sperm Quality of Barbados Blackbelly Rams under Different Management Systems in the Tropics (manuscript in prep)

HORMONAL RESPONSE

- **Similar testosterone levels** indicate lack of impact by type of management system.
- In line with behavioural data: **testosterone concentration relates to sexual behaviour** (Maksimović et al., 2021).
- **Average cortisol levels in SIS was higher, but not significant.** HS activates the hypothalamic-pituitary-adrenal (HPA) and sympatho-adrenal-medullary (SAM) systems which later **stimulates the production of glucocorticoids** such as cortisol (Barragán Sierra et al., 2021).

REPRODUCTIVE PERFORMANCE

Semen volume and sperm concentration of rams in different management sysems

- HS did not result in significant difference in sperm concentration and semen volume, implying that **mature sperm are not affected** (Barragán Sierra et al., 2021; Rizzoto & Kastelic, 2020; El Amiri, 2020).

Mazlishah et al. Influence of the Temperature-Humidity Index (THI) on the Sperm Quality of Barbados Blackbelly Rams under Different Management Systems in the Tropics (manuscript in prep)

REPRODUCTIVE PERFORMANCE

Sperm viability and morphology abnormalities of rams in different management systems

- **Higher THI** result in **higher sperm abnormalities** (SIS > ITS).
  - Long exposure to **HS result in major and minor abnormalities** (Moula et al., 2024).
  - Damage to the germ cells **during spermatogenesis** could be the cause of the abnormalities seen (Van wetter et al., 2021).
  - Detrimental **alterations in the spermatocyte meiosis cycle** during the spermiogenesis process result in impairments of sperm morphology during heat stress (Barragan et al., 2024).

- **Developing sperm affected during spermatogenesis (49 days) and remain in the epididymis until ejaculation.**

Mazlishah et al. Influence of the Temperature-Humidity Index (THI) on the Sperm Quality of Barbados Blackbelly Rams under Different Management Systems in the Tropics (manuscript in prep)

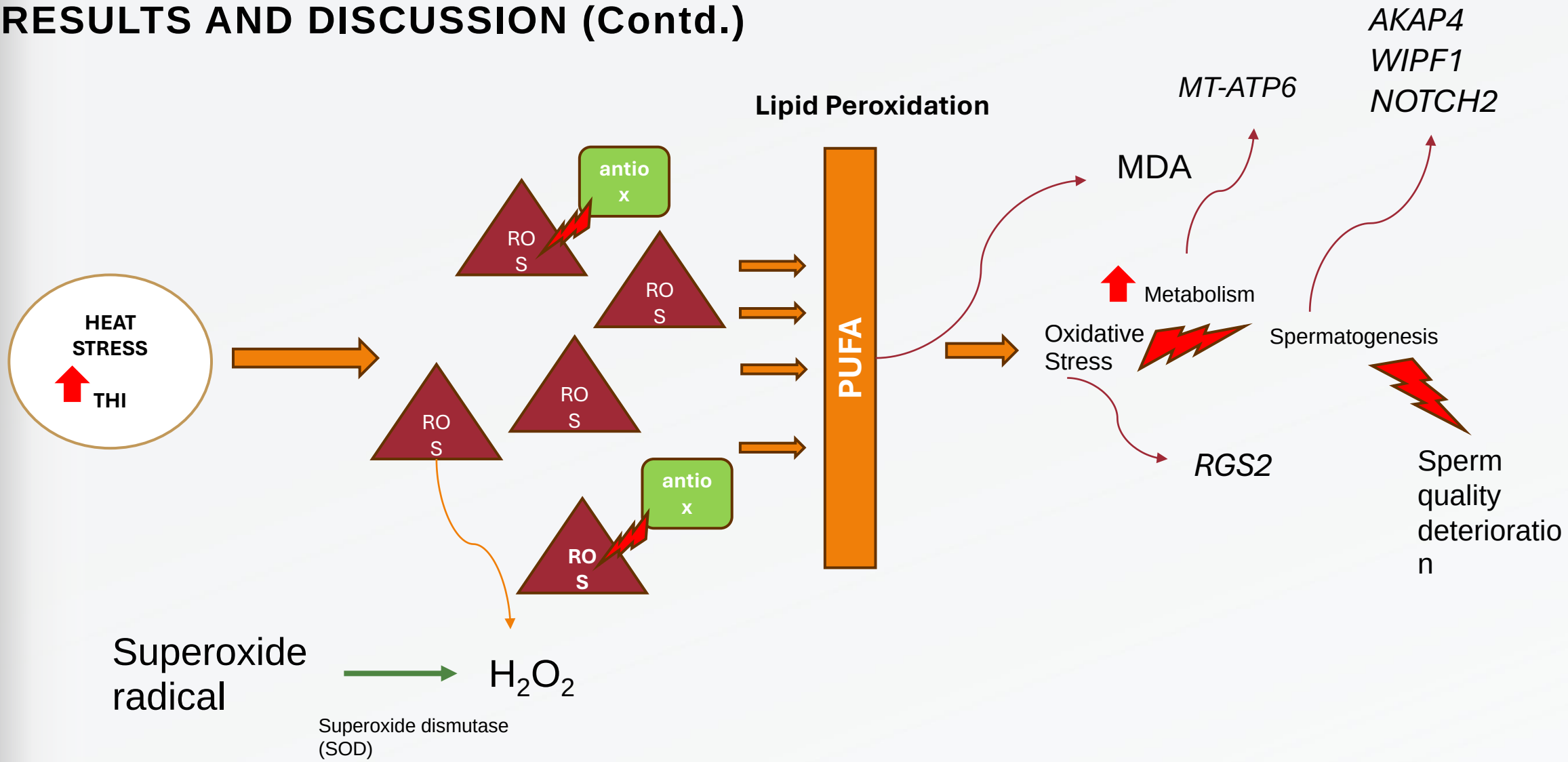
OXIDATIVE STRESS

- **MDA** is an indicator **of lipid peroxidation** (Ahmad Jamil & Abdul Karim, 2024).
- Exposure of heat stress increases MDA concentration, indicating increase in oxidative stress (Ijiri et al., 2022). **Parallels the observed deterioration in sperm motility and morphology.**
- MDA in both management systems **still within fertile ranges below, 3 nmol/mL** (Almady et al., 2021).

OXIDATIVE STRESS

- H_2O_2 is a ROS and marker of oxidative stress. Balanced H_2O_2 concentration is crucial to ensure **maturation of spermatozoa, stability of chromatin, sperm hyperactivation, acrosome reaction, and fertilization rate** (Aitken et al., 1995; Qamar et al., 2023)
- **Average H_2O_2 concentration** averaged across all weeks were **higher in SIS**, in line with other parameters such as sperm quality and physiological assessments.

RESULTS AND DISCUSSION (Contd.)



THI | **Oxidative Stress** | **Gene expression** | **Sperm parameters**

CONCLUSION

01 Rams in semi-intensive system were exposed to higher THI and exhibited significant physiological responses and sperm quality deterioration.

02 OS data support the occurrence of heat stress in semi-intensive rams.

It can be suggested that **rams under semi-intensive systems may show lower fertility** compared to those in intensive systems. However, the **semi-intensive system does not severely affect reproductive performance to the extent of causing infertility**. The negative effects on reproductive performance remain within an acceptable range.

Therefore, it can be proposed that the semi-intensive system can still be practiced, provided that appropriate mitigation strategies are implemented to minimize the impact of heat stress.

Key takeaways

- High THI affects sperm quality (viability, motility, abnormality)
- HS-induced OS, more prominent in long-term high THI exposure.
- Evidence for tropical adaptability of Barbados Blackbelly?
    - No significant differences in cortisol and testosterone a sign of rapid acclimation to high THI
- Current THI indices may not be suitable for the tropics and across all small ruminant breeds, such as Barbados Blackbelly sheep.
    - There is a need for THI vs. thermal comfort indices to be developed for small ruminants in the tropics.
    - Based on our data, respiration rate and testicular temperature could be reliable stress indicators for BB sheep.

Ongoing and future work

- Gene expression analysis (qRT-PCR and RNAseq) from sperm transcriptome
- Stress recovery
- Female reproductive characteristics
- Long-term semi-intensive rearing vs. reproductive performance
    - Annual meteorological data

Acknowledgements

- Mohd Shahmi Hakimi Mazlishah
- Muhammad Syafiq Roslan
- Noor Hashida Hashim
- Nor Azlina Abd. Aziz
- Mohd Fadzil Firdzaus Mohd Nor
- Noorhidayah Mamat
- Postgraduate students, interns, and staff at the Glami Lemi Biotechnology Research Centre, Universiti Malaya (PPBGL)

Funding

Fundamental Research Grant Scheme, Ministry of Higher Education Malaysia