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Stress analysis due to semen collection using infra-red thermography in rabbits

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ABSTRACT

This research evaluated the thermal response to semen collection in rabbits using infra-red thermography in the eyeball, pinna, and nose, alongside measurements from an infra-red forehead thermometer on the pinna. In the first experiment, 20 rabbits were divided into control and stressed groups. Basal temperatures were recorded at minutes 0, 1, 5, and 10, with semen collected at minute 0 for the stressed group. The stressed group showed a temperature rise in the eyeball at minute 5 (37.80 °C vs. 37.58 °C; $p = 92\%$), but no in the nose or pinna ($p < 90\%$). The thermometer provided results consistent with IRT for the pinna. In the second experiment, 40 rabbits underwent temperature monitoring over 30 min, with semen collection at minute 0. Rabbits were then divided into single- and double-stressed groups. For the double-stressed group, a second semen collection occurred at minute 30, with measurements until 90 min. Eyeball temperatures rose between minutes 0 and 1 (36.37 °C–36.61 °C; $p = 99\%$) and stabilised by minute 30 ($p = 94\%$). A second reaction to semen collection was observed between minutes 30 and 40 ($p = 93\%$), but baseline temperatures were not restored. In the pinna and nose, thermal reactions were identified within the first 15 min ($p > 99\%$) but also failed to stabilise. These findings confirm that semen collection induces stress, with the eyeball providing the fastest and most stable thermal response. Infra-red thermography is a reliable, non-invasive monitoring tool, and infra-red thermometers offer a cost-effective alternative for the pinna.

HIGHLIGHTS

- Semen collection can be a stressful stimulus that triggers a thermal reaction.
- Infra-red thermography is a minimally invasive and effective method for measuring temperature changes.
- The eyeball is the most suitable anatomical region for measuring stress-related temperature changes in the rabbit. This finding enhances the acquisition of knowledge in the welfare of this pet, productive and laboratory animal

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Buck; infra-red thermography; welfare; eyeball; semen collection

Introduction

Breakthroughs in livestock science and technology have expanded our understanding of animal welfare. Stress is defined as their response to environmental stimuli that disrupt homeostasis (Palumbo et al. 2020). Rabbit (*Oryctolagus cuniculus*), due to their late domestication, retain many wild behaviours (Naff and Craig 2012), making them particularly sensitive to stress (Jenkins 2001). Their shy nature complicates the observation of welfare changes (Jenkins 2001).

Stress from handling or transport can lead to metabolic and immune disorders (Verwer et al. 2009; María et al. 2010) that negatively impact productivity (Jaén-Téllez et al. 2021), with physical suffering (Cheng et al. 2001) and disease susceptibility (Glaser and Kiecolt-Glaser 2005). Identifying stress accurately is thus crucial. Stress triggers the hypothalamic-pituitary-adrenocortical axis and the sympathetic adrenomedullary system, increasing glucocorticoids (cortisol and corticosterone) and catecholamines (adrenaline and noradrenaline) (Hennessy 1997; Möstl and Palme 2002). Elevated cortisol stimulates glycogenolysis and gluconeogenesis, raising glucose levels and reducing insulin levels (Shaw and Tume 1992; Składanowska-Baryza et al. 2018; Urbanová et al. 2019). Therefore, cortisol has been traditionally used as a biomarker in stress (de Prada et al.

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2007; Argente et al. 2014). Recently, testosterone, triglycerides, cholesterol and acute phase proteins have been proposed as useful alternatives to cortisol (Tsopanakis et al. 1988; Lata et al. 2004; Eckersall and Bell 2010; Argente et al. 2014; Habeeb et al. 2018). Besides, stress also causes neutrophilia and lymphocytopenia, with the neutrophil-to-lymphocyte ratio serving as an alternative measure (Widowski et al. 1989). These biomarkers' collection methods can be invasive and their levels variable. Cortisol levels fluctuate daily (Ruis et al. 1997) and sampling itself can be stressful, subsequently modifying the release of cortisol as a consequence of the manipulation of the animal (Hopster et al. 1999). Studying gut microbiota (brain-gut-microbiota axis) offers another option (Dinan and Cryan 2012; Feng et al. 2022), but it often requires sacrificing the animal (Fu et al. 2018).

Non-invasive stress assessment methods include measuring catecholamine and glucocorticoid levels in urine (Hay and Mormède 1998) and detecting cortisol in saliva (Kirschbaum and Hellhammer 1989; Hellhammer et al. 2009), faeces (Möstl and Palme 2002) and hair samples (Peric et al. 2018). Haematophagous insects can also be used (Voigt et al. 2004). Increased intraocular pressure is another stress indicator (Miyazaki et al. 2000).

Stress-induced catecholamine secretion increases heart activity and splenic contractions, raising red blood cell counts (Axelrod and Reisine 1984; Collin et al. 2001). This boosts blood flow, heat production and heat loss (de Lima et al. 2013). Through the temperature radiated by the skin we can assess the physiological state of animals (Alsaaod et al. 2014; Cook et al. 2015). Infra-red thermography (IRT) is an effective non-invasive method that detects body temperature changes through radiated electromagnetic energy (Stewart et al. 2005; Tattersall 2016; Unruh et al. 2017; Wongsaengchan et al. 2023). Recently, IRT has been applied in rabbit farming, specifically in breeding females and fattening males (Agea et al. 2021; Jaén-Téllez et al. 2021). Temperature can be measured in several anatomical regions. The rabbit is a homeothermic animal without sweat glands and relies on its ears and nose to dissipate heat (Caputa 1979; Lebas et al. 1997). This makes these regions suitable for thermographic assessment (de Lima et al. 2013). The eyeball has also been reported as a good region for assessing changes in temperature (Jaén-Téllez et al. 2020).

Artificial insemination is a highly efficient assisted reproductive technology that has become common practice in rabbit farms (Viudes-de-Castro and Vicente, 2023). Semen collection in artificial insemination could cause additional stress to natural mating. Therefore, while ejaculation is a normal physiological function, artificial semen collection involves handling and restraint, which are factors that can elicit a stress response in animals, as previously reported by Fazio et al. (2017) and Olivas and Villagrá (2013), and it could affect semen quality.

We hypothesised that semen collection is a stressor with the thermal response and that the eyeball is an anatomical region less subject to environmental changes and, therefore, suitable for assessing stress-related temperature changes. The aims of this study were to assess the thermal stress response by IRT after semen collection as a stressor and to determine the most appropriate anatomical region and moment for its measurement in the rabbit.

Material and methods

Animals and experimental design

The research was carried out on the farm at Miguel Hernández University in Orihuela, Alicante (Spain). The facilities used were a controlled environment (11 °C–24 °C) and 16 L:8D photoperiod. Animals were housed in individual cages and fed with a commercial diet (16.1% of crude protein; 3.522 kcal/kg of digestible energy) and provided water *ad libitum*. Males belonging to synthetic lines were used (Blasco et al. 2017). Age ranged between 5 and 7 months. The average weight was 3.46 kg.

Males started the training period at 150 days of age. The training was performed for 2–3 weeks (Lavara et al. 2011). After the training, the males entered in the production period. Data collection was carried out for nine weeks, between February and June 2023. Two procedures were used to measure the temperature of the males:

1. Body temperature emissivity was measured using IRT on the eyeball, pinna and nose, inside the farm (Figures 1–3). The images were obtained using a ®FLIR SC660 thermal imaging camera. The images were processed with the ®ThermaCAM Researcher Pro 2.10 software to obtain the temperature record. The camera was calibrated according to temperature, relative humidity, emissivity (98%) and distance from the subject (0.7 m).
2. The temperature of the pinna was also collected with an infra-red forehead thermometer ®iHetal PT3. The temperature was taken at 3 cm from the pinna.

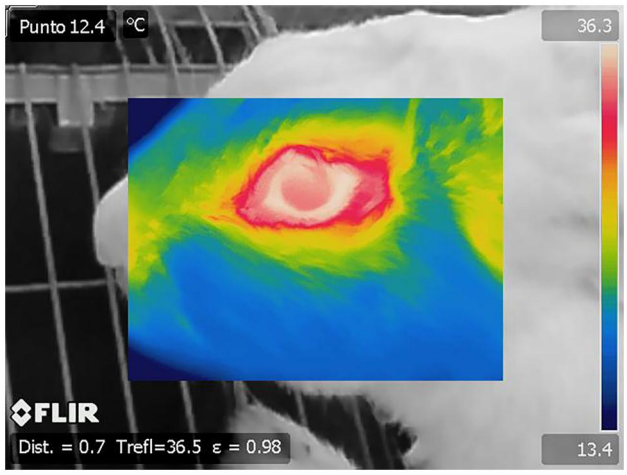

Figure 1. Infra-red image of the eyeball in a male rabbit taken with the ®FLIR SC660 thermal imaging camera. Dist: distance; trefl: Reflected temperature; ε: emissivity.

Figure 2. Infra-red image of the nose in a male rabbit taken with the ®FLIR SC660 thermal imaging camera. Dist: distance; trefl: Reflected temperature; ε: emissivity.

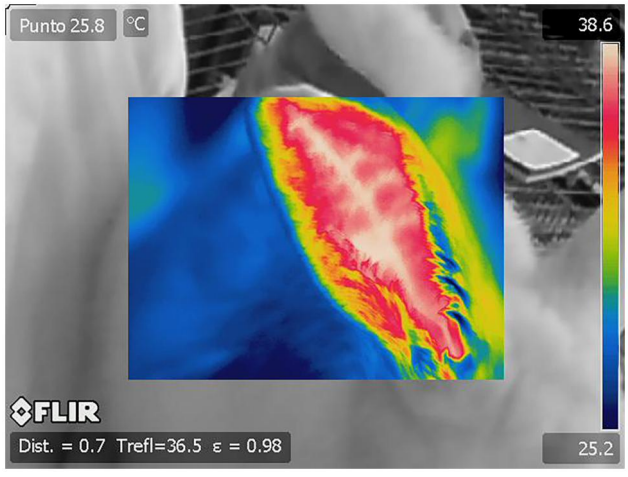

Figure 3. Infra-red image of the pinna in a male rabbit taken with the ®FLIR SC660 thermal imaging camera. Dist: distance; trefl: Reflected temperature; ε: emissivity.

First experiment

The first experiment was designed to find out whether semen collection produced additional stress to the male manipulation carried out for temperature recording. A total of 20 males were used (10 control group males and 10 from the stressed group). The experimental procedure was carried out early in the morning. The animals were held by a specialist technician from the top of the back, without touching the eyes, nose and ears, to take temperature measurements. First, the basal temperature was taken. Semen was then collected with the rabbit inside their cages, and the temperature was measured at 1, 5 and 10 min. The stressor was semen collection with an artificial vagina tempered to 45 °C (Boiti et al. 2005). No semen collection was performed in the control group.

Second experiment

The second experiment was designed to determine the most appropriate place and time to record thermal reaction to a stressful stimulus. A total of 40 males were used (20 males in the single stress group with one semen collection and 20 males in the double stress group with two semen collections). Measurements were taken at 15 time points (minutes 0 or basal temperature, 1, 5, 10, 15, 20, 25, 30, 31, 35, 40, 50, 60, 70 and 90). Semen collection was performed at minute 0 in both groups and at minute 30 in the double-stressed group. From minute 0–30, both groups were considered together, which we have called the common path.

Statistical analysis

To assess the difference between groups and time in IRT on the eyeball, pinna and nose and pinna temperature with a thermometer, the following model was used (as there are repeated measurements of the same male, the random effect of the male was included in the model):

$$y_{ijkl} = \mu + G_i + T_j + (G \times T)_{ij} + b^{*}W_{ijk} + m_{ijk} + e_{ijkl},$$

where G_i is the group effect ($i = 2$; control and stressed group), T_j is the time effect ($j = 4$; 0 or basal temperature, 1, 5 and 10 minutes), $(G \times T)_{ij}$ is the interaction group and time (8 levels), b is the regression coefficient, W_{ijk} is the covariate weight, m_{ijk} is the male effect and e_{ijkl} is the residual term.

To assess the difference between groups in the common path, single-stressed group and double-stressed group for the eyeball temperature recorded with IRT, the following model was used:

$$y_{ijkl} = \mu + G_i + T_j + (G \times T)_{ij} + b^*W_{ijk} + m_{ijk} + e_{ijkl},$$

where G_i is the group effect (i = 3; stressed common path, single-stressed group and double-stressed group), T_j is the time effect (j = 15; minutes 0 or basal temperature, 1, 5, 10, 15, 20, 25, 30, 31, 35, 40, 50, 60, 70 and 90), $(G \times T)_{ij}$ is the interaction group and time (22 levels), b is the regression coefficient, W_{ijk} is the covariate weight, m_{ijk} is the male effect and e_{ijkl} is the residual term.

All analyses were performed using Bayesian methodology. Bounded uniform priors were used for all effects except for the male effect, considered normally distributed with mean 0 and variance $I\sigma^2_p$. Residuals were a priori normally distributed with mean 0 and variance $I\sigma^2_e$. The priors for the variance were also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Inferences were derived from the marginal posterior distributions. Means, standard errors and actual probability (P) were provided. P refers to the probability that the absolute value of the difference between two levels of a fixed effect will be greater than zero (Blasco et al. 2017). We consider that there are relevant differences when the P is greater than or equal to 90%. The Rabbit software program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures (more details about its features are given at http://www.dcam.upv.es/dcia/ablasco/Programas/THEPROGRAMRabbit.pdf). We used a chain of 60,000 samples, with a burn-in period of 10,000. Only one out of every 10 samples was saved for inferences. Convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures.

Results

First experiment

Figure 4 shows the means and standard errors of the control and stressed group for the eyeball temperature recorded with IRT. The temperature increased between minutes 0 and 1 for the control group (37.32 and 37.61 °C; $p = 99\%$) and stressed group (37.32 °C and 37.64 °C; $p = 99\%$). The stressed group shows a tendency to increase its temperature until minute 5 (37.80 °C; $p = 85\%$), while the control group kept the temperature constant until minute 10. Moreover, the control group showed a lower temperature than the stressed group at minute 5 ($p = 92\%$). Temperature decreased between minutes 5 and 10 in the stressed group (37.58 °C; $p = 94\%$).

Control and stressed groups did not react between minutes 0 and 1 ($p = 64\%$, Figure 5). Temperature was higher at minute 5 than minute 1 for both groups ($p > 90\%$). Between minutes 5 and 10, the control group temperature continued to rise (37.00; $p = 90\%$), but the stressed group temperature did not ($p = 68\%$).

The temperature measured with the thermometer was always lower than the temperature measured by IRT, with the difference between the devices ranging from 0.26 °C to 0.78 °C, although the temperature evolution was similar for both devices (Figure 6). Regarding the measurements taken with IRT, the

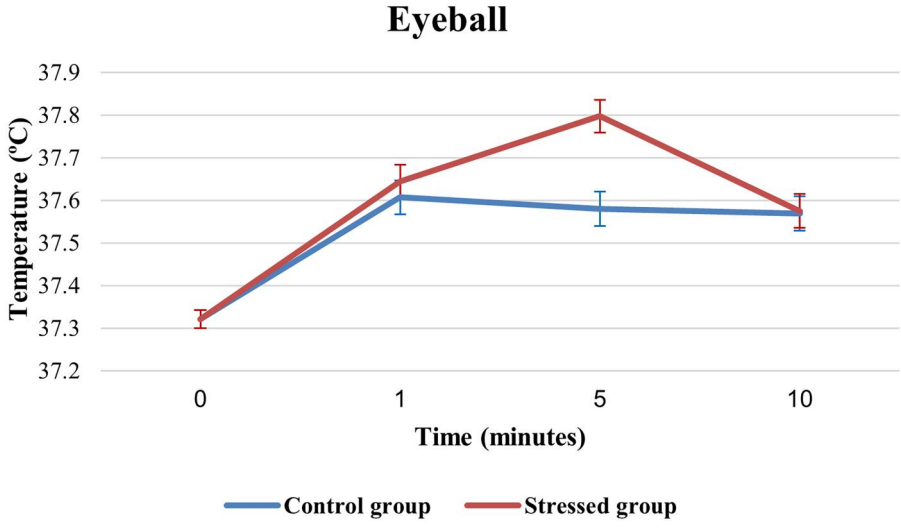

Figure 4. Means and standard errors (bars) of marginal posterior distributions of the control group and stressed group for the eyeball temperature recorded with infra-red thermography.

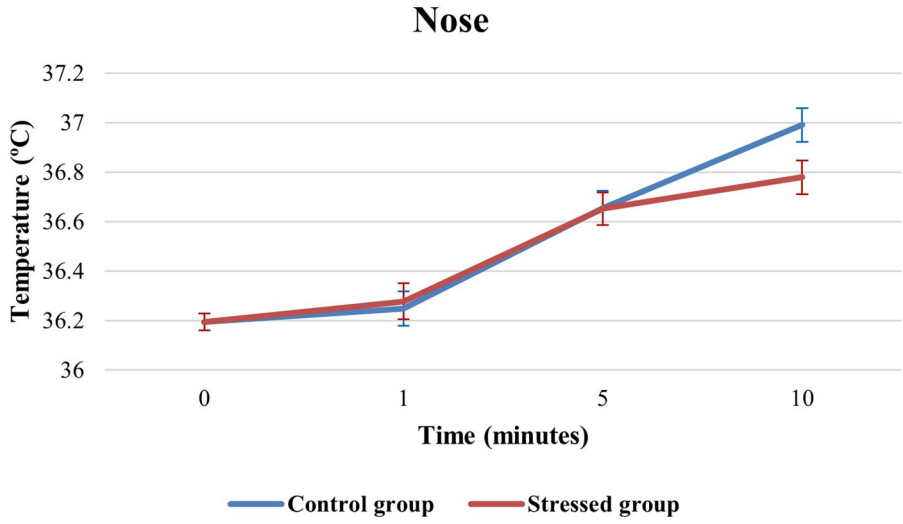

Figure 5. Means and standard errors (bars) of marginal posterior distributions of the control group and stressed group for the nose temperature recorded with infra-red thermography.

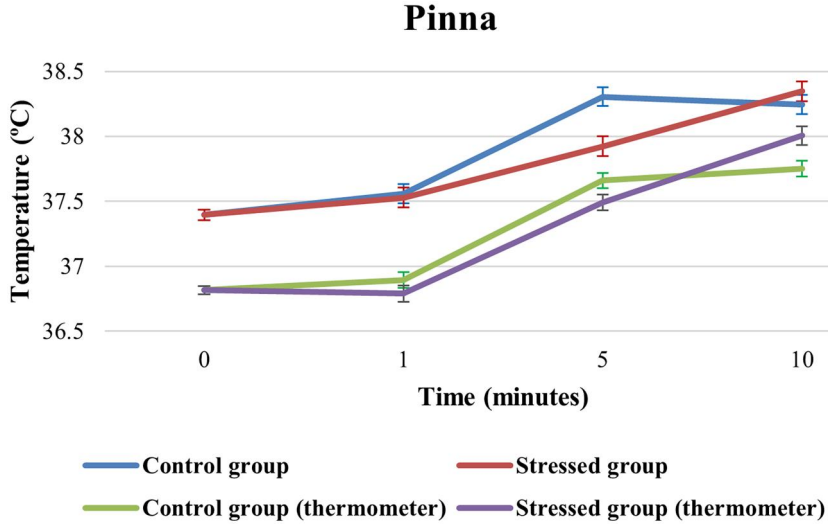

Figure 6. Means and standard errors (bars) of marginal posterior distributions of the control group and stressed group for the pinna temperature recorded with infra-red thermography and thermometer.

control group and the stressed group did not respond to the stimulus between minutes 0 and 1 ($p = 77\%$ and $p = 72\%$, respectively). The response was located between minutes 1 and 5 for control group (37.56 °C and 38.30 °C; $p = 99\%$) and the stressed group (37.53 °C and 37.92 °C; $p = 95\%$). After minute 5, while the stressed group increased its temperature (38.35 °C; $p = 95\%$), the control group temperature was maintained ($p = 55\%$). At minute 5, a temperature difference was observed between both groups ($p = 91\%$).

When the thermometer was applied, the reaction was detected between minutes 1 and 5 in both groups (36.89 and 37.66; $p = 99\%$ for the control group; 36.79 °C and 37.49 °C; $p = 99\%$ for the stressed group). Between minutes 5 and 10, the stressed group increased its temperature (38 °C; $p = 98\%$), but the control group did not ($p = 64\%$).

Second experiment

Figure 7 shows the means and standard errors of the common path, single-stressed group and double-stressed group for the eyeball temperature recorded with IRT. The reaction to the stressful stimulus occurred between minute 0 and 1 (36.37 °C and 36.61 °C; $p = 99\%$). Between minutes 1 and 5 the temperature decreased (36.47 °C; $p = 94\%$). From minute 5 it remained constant until minute 30 (36.32 °C; $p = 94\%$).

Between 30 and 31 minutes, the double-stressed group reacted by increasing its temperature (36.57 °C;

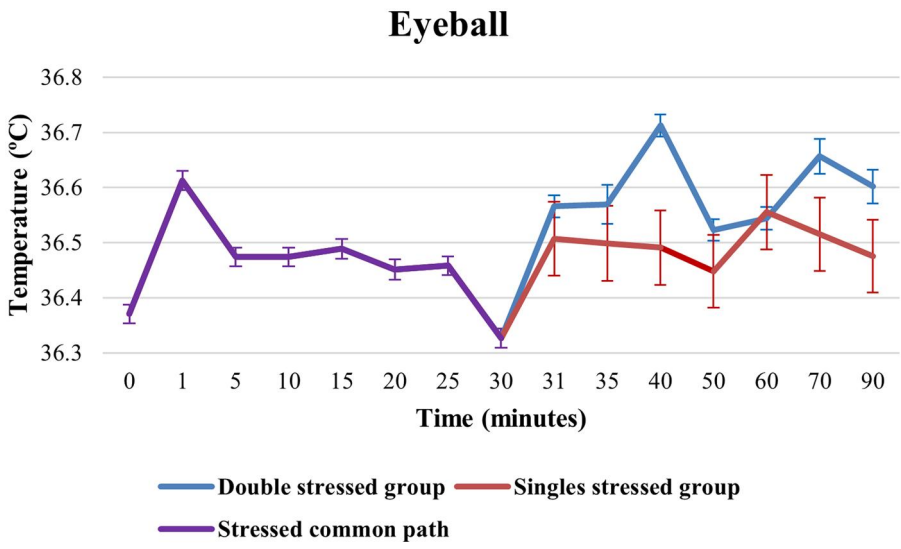

Figure 7. Means and standard errors (bars) of marginal posterior distributions of the common path, single-stressed group and double-stressed group for the eyeball temperature recorded with infra-red thermography.

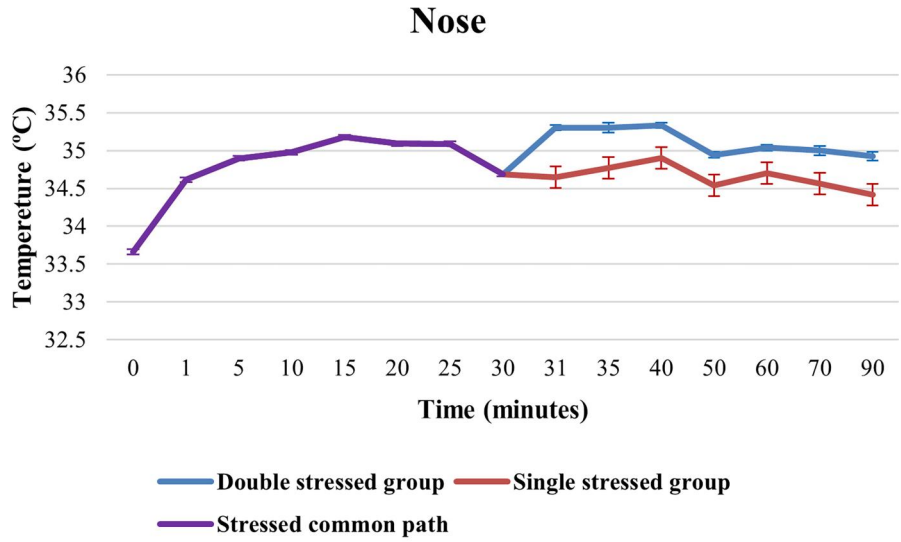

Figure 8. Means and standard errors (bars) of marginal posterior distributions of the common path, one semen collected group, two semen collected group for the nose temperature recorded with infra-red thermography.

$p = 99\%$). The temperature increased until minute 40 (36.71 °C; $p = 93\%$). From 40 minute, the temperature decreased until 60 minute (36.54 °C; $p = 95\%$). Between 60 and 90 minutes, temperature was similar ($p < 82\%$).

The single-stressed group showed a tendency to increase temperature between minutes 30 and 40 (36.32 °C and 36.49 °C; $p = 80\%$). It subsequently remained stable ($p < 80\%$). Both groups showed differences only at minute 40 ($p = 91\%$)

The reaction to the stimulus took place between 0 and 1 minutes (33.66 and 34.61 °C; $p = 100\%$, Figure 8). The temperature continued to increase until 15 minute (35.17 °C; $p = 99\%$) and remained constant until 25 minute ($p = 66\%$). Between 25 and 30 minutes, the temperature decreased (35.09 °C and 34.69 °C; $p = 99\%$).

The double-stressed group showed the reaction to the second stimulus between 30 and 31 minutes (35.30 °C; $p = 100\%$). The temperature remained constant until minute 40 ($p < 90\%$). Between 40 and 50 minutes, the temperature decreased (35.33 and 34.94 °C; $p = 95\%$) and remained constant until minute 90 ($p < 80\%$).

The temperature of the single-stressed group remained constant throughout the series ($p < 80\%$). Both groups showed differences between 31 and 35 minutes ($p > 90\%$).

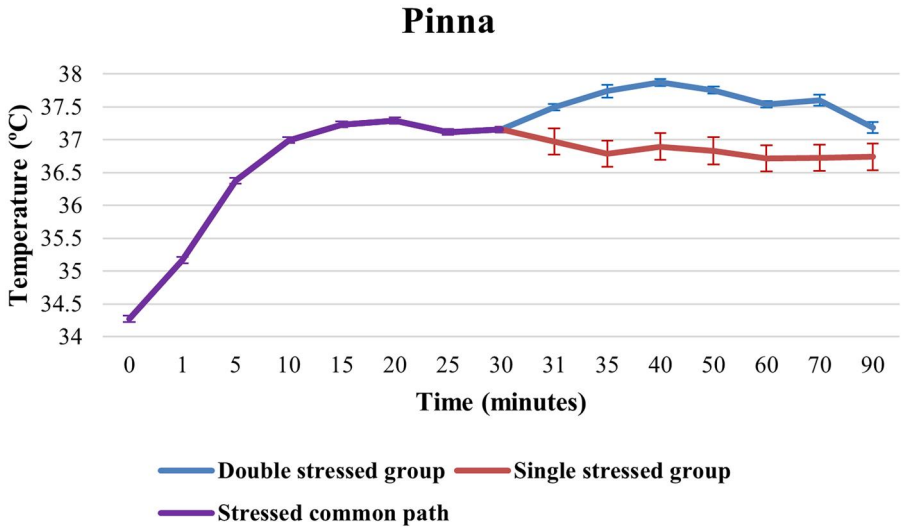

Figure 9. Means and standard errors (bars) of marginal posterior distributions of the common path, single-stressed group and double-stressed group for the pinna temperature recorded with infra-red thermography.

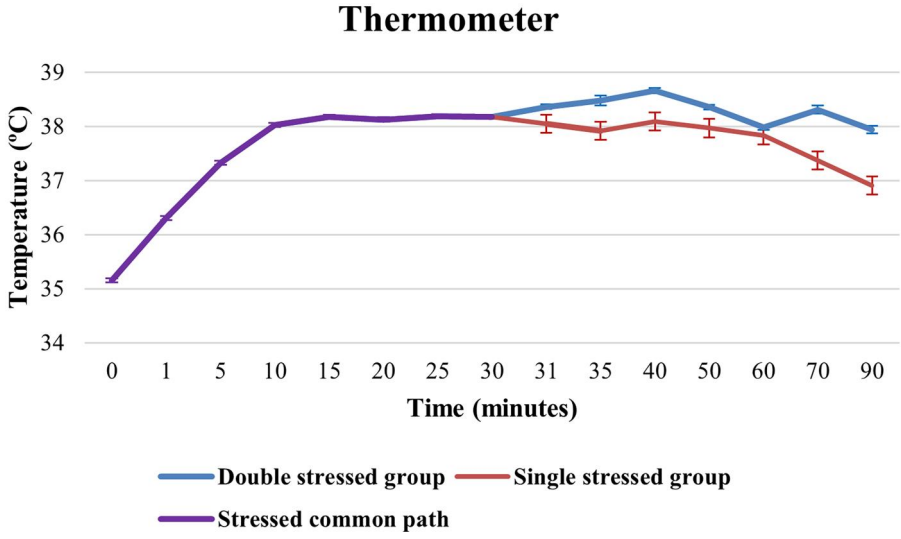

Figure 10. Means and standard errors (bars) of marginal posterior distributions of common path, one semen collected group, two semen collected group control group and stressed group for the pinna temperature recorded with thermometer.

The temperature increased from minute 0 to 15 (34.27 °C and 37.23 °C; $p = 100\%$) then stabilised until 30 minute (37.16 °C; $p = 66\%$, Figure 9).

After the second stimulus, the reaction occurred during the next 5 min (37.74 °C; $p = 92\%$). In the following minutes, the temperature remained constant until minute 90, where a decreasing was observed (37.19 °C; $p = 90\%$).

In the single-stressed group, the temperature remained stable until minute 90 (36.74 °C; $p = 75\%$). From 35 to 70 minute, the double-stressed group showed a higher temperature than the single-stressed group ($p > 90\%$).

The thermal reaction to the stimulus measured with the thermometer in the pinna was located between minutes 0 and 1 (35.15 °C; 36.30 °C; $p = 100\%$, Figure 10). The temperature continued to increase until minute 15 (38.18 °C) and remained stable until minute 30 (38.17 °C; $p = 52\%$).

The second reaction took place between 30 and 40 minutes (38.66 °C; $p = 97\%$). The temperature decreased between 40 and 60 minutes (37.98 °C; $p = 100\%$). Temperature was constant after 60 minute.

Temperature remained constant ($p < 90\%$) until minute 60, then decreased until 90 minute for the single-stressed group (36.91 °C; $p = 99\%$). The double-

stressed group showed higher temperature than the single-stressed group at 70 and 90 minutes ($p > 90\%$).

Discussion

Stress causes physiological variations that result in alterations in blood flow and changes in body temperature (Schaefer et al. 2002). Thus, IRT is a useful tool to assess these changes in a non-invasive way (Nääs et al. 2014).

In the rabbit, the three main regions for temperature assessment by IRT are the pinna, the nose and the eye. As in our experiment, de Lima et al. (2013) reported differences between the temperatures of the three regions. It should be noted that not all anatomical regions of an animal emit the same amount of infra-red radiation (McManus et al. 2016).

Rabbits are restless animals, which makes it difficult to take thermographic images without handling them. The first experiment aimed to assess the existence of differences between handling the rabbit and handling plus a semen collection as the main and routine stressful stimulus on the rabbit farm. In the eyeball, the temperature of the stressed group increased longer than in the control group. The highest temperature difference between the two groups was 0.22 °C ($p = 92\%$) at minute 5. Thus, this result confirms that semen collection is a stressor and can be identified by IRT. Jaén-Téllez et al. (2020) also identified a rapid increase in temperature in the eyeball when fattening rabbits are stressed by handling.

The temperature collected from the pinna or nose does not seem to have been able to identify the stress caused by semen collection. Thus, in the nose, changes of temperature were similar between the two groups and no differences were found between the maximum temperatures reached. Regarding the pinna temperature, the difference between the control and stressed groups was found at minute 5 (0.38 °C; $p = 91\%$). However, from minute 5 onwards the temperature of the control group stabilised, while that of the stressed group continued to rise until it reached the same temperature. The temperature recorded by the thermometer showed a similar pattern but no differences between the two groups ($p = 64\%$). In all cases, an increase in body temperature is produced. This phenomenon is known as *stress-induced hyperthermia* (Bouwknecht et al. 2007), however, it is the semen collection stimulus that produces a different response.

In the second experiment, the reaction to semen collection in the eyeball was identified between minutes 0 and 1. Since in the first experiment the reaction was shown between minutes 1 and 5, this leads us to believe that the temperature increase in the eyeball is localised in the first five minutes after a stressful stimulus. From the sympathetic nervous system, the hypothalamus stimulates the adrenal glands to secrete catecholamines (adrenaline and noradrenaline) (Cunningham 1997; Duval et al. 2010). In the rabbit, this reaction occurs within seconds, causing a rapid rise in body temperature (Manteca 1998; Bouwknecht et al. 2007). The metabolism of these hormones is very fast (McCarty 1983): their half-life is only a few minutes, so it is a short-lived response (Ginn and Vane 1968; Peaston and Weinkove 2004). Consistent with the rapid metabolism of catecholamines, after a few minutes the temperature in the eyeball dropped to basal temperature. From this point on, there would be an increase in cortisol levels from the adrenal cortex proportional to the severity of the stress, called the *general adaptation syndrome* (Gascón and Arribas 1987; Cunningham 1997).

In rabbit farming, the seminal collection is usually performed twice on the same day with a separation of 30 min for each collection (Lavara et al. 2005). In the eyeball, after 30 minute, both groups showed a thermal reaction in the first minute (30–31 minutes). However, the double-stressed group continued to increase its temperature until minute 40. These results are consistent with those described in the first experiment, in which both groups increased their temperature, but semen collection favoured a greater increase. While after the first ejaculation the highest temperature occurs within the first minute, after the second ejaculation the highest temperature occurs after ten minutes. This may be due to negative feedback in the production of tyrosine (adrenaline precursor) by the adrenaline itself after the first ejaculation (Hedge et al. 1987). However, adrenaline metabolism is very fast and does not necessarily have to be the cause. Another explanation is the animal's own adaptation to the second seminal collection, resulting in a less acute response to the stressful stimulus than the first time. Neither group was able to return to their basal temperature. Thus, 90 min is not enough to recover from the stressor stimulus.

In the pinna and the nose, the thermal reaction to the stressful stimulus is later than in the eyeball, and it occurs during the first 20 and 15 min, respectively. The delayed reaction in the pinna may explain the differences found in the first experiment. While from minute 5 onwards the control group stabilised, the stressed group continued to increase its temperature beyond minute 10.

In contrast to the eyeball, the temperature did not stabilise with respect to the basal temperature at any time during the experiment in both regions. The pinna and the nose, together with the abdominal surface, are the two main regions of heat dissipation in the rabbit (Harada 1971; Caputa et al. 1980). In addition, the temperature of the nose can vary according to the humidity of the environment (Luzi et al. 2007). As these are regions for heat exchange, it is to be expected that the highest temperature is reached later than in the eyeball and does not decrease to basal temperature for a long period of time. After minute 30, the single-stressed group continues to slowly decrease in temperature (without reaching the basal temperature) both in the pinna and in the nose. In the double-stressed group, a reaction takes place again, but with a lower temperature increase than after the first semen collection.

In other ways, de Lima et al. (2013) and Jaén-Téllez et al. (2020) also came to the conclusion that the eye might be the most suitable anatomical region for thermal stress control. Its rapid response and stabilisation make the eyeball ideal for simple and precise monitoring.

Kukkonen et al. (2010) showed an increase of about 1 °C during sexual arousal in male sexual organ. However, our findings indicated temperature increases in other anatomical regions, such as the ears, eyes, and nose, which are consistent with sympathetic nervous system activation and a physiological stress response (Pasing et al. 2013). Furthermore, the observed thermal response follows a pattern consistent with stress-induced hyperthermia, a phenomenon that has been extensively documented in rabbits subjected to handling (Villegas-Cayllahua et al., 2022).

We consider that the assessment of thermal responses using infra-red thermography provides valuable insights into the physiological changes associated with semen collection, reducing potential bias from human contact and allowing for objective measurement (Nääs et al. 2014).

It is worth noting that the temperature recorded by the thermometer in the pinna showed a similar evolution to the thermography, although with a lower temperature. Considering this difference in accuracy, the thermometer could be a valid and much cheaper tool for routine monitoring of stress response on a commercial farm.

Finally, some authors point out that an animal's previous experience in the seminal collection may favour a lower stress response (Pasing et al. 2013). In this respect, we would like to point out that the males were subjected to a training period to get used to the use of the artificial vagina. Therefore, it is to be expected that although young adult males have been used, the results can be extrapolated to older males.

Conclusions

In rabbits, the seminal collection is a stressful stimulus that is reflectetobody temperature. IRT is a suitable and minimally invasive tool for measuring body temperature. Among the main anatomical regions for measuring body temperature in rabbits by IRT, the eyeball offers the fastest response and stabilisation to basal temperature. The eyeball is an ideal region to study the evolution of body temperature under an acute stress stimulus quickly and easily. The infra-red forehead thermometer is an effective and low cost, though less accurate, tool for assessing temperature changes in the pinna.

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Authors contributions

Daniel Serrano-Jara: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **Iván Agea**: Conceptualisation, Methodology, Writing - review & editing; **José Ramón Díaz**: Conceptualisation, Methodology; **María José Argente**: Conceptualisation, Data curation, Formal analysis, Investigation, Supervision, Methodology, Writing - review & editing; **María de la Luz García**: Conceptualisation, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - review & editing

Ethical approval

The experimental procedures with animals were approved by the General Directorate of Agriculture, Livestock and Fisheries of the Generalitat Valenciana with code 2022/VSC/PEA/0226.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

None of the data were deposited in an official repository. All data are available upon request.

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