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Thermal gradient effects on motility and viability of ram sperm

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ABSTRACT. Sperm thermotaxis, the directed movement of sperm along temperature gradients, helps spermatozoa to navigate the female reproductive tract. Although this phenomenon has been studied in many species, its relevance in livestock, particularly in rams, remains to be explored. The present study aimed to determine *in vitro* thermotactic behaviour of ram spermatozoa and its impact on sperm concentration, motility and viability across different temperature gradients. Sperm collected from a mature Awassi ram was subjected to a swim-up procedure and incubated for 60 min in two thermal gradient tubes consisting of two compartments maintained at 33 °C and 41 °C, respectively. An isothermal tube with two compartments maintained at 38 °C served as the control. Sperm concentration, kinetic parameters, membrane integrity, and viability were assessed in each compartment. No significant differences were observed between the compartments of the isothermal control. In contrast, sperm concentration was

significantly higher in the cooler compartment (33 °C) than in the warmer (41 °C) compartment of the thermal gradient group. Motility also tended to be higher at 33 °C (P = 0.054). Although some velocity parameters differed numerically between temperature conditions, these differences were not statistically significant. Exposure to 41 °C markedly reduced sperm viability compared with both the initial semen sample and the cooler compartment. These findings demonstrate that ram spermatozoa show thermotactic behaviour *in vitro* and preferentially accumulate in cooler regions. This response is also associated with improved motility, membrane stability and viability. These findings suggest that thermotaxis may contribute not only to sperm navigation but also to the selection of spermatozoa with higher motility and viability in small ruminants.

KEY WORDS: ram sperm, sperm motility, thermotaxis, viability

Introduction

In mammals, successful fertilisation depends on the ability of the spermatozoa to locate the oocyte in the ampulla of the oviduct. After ejaculation, sperm pass through the cervix, uterus before crossing the uterotubal junction and entering the oviduct, where they encounter biophysical barriers because of their small size (Eisenbach and Tur-Kaspa, 1999; Bahat and Eisenbach, 2006; Hoang and Miller, 2017). Successful passage through the female reproductive tract requires sustained sperm motility. However, motility alone does not fully explain how a small proportion of spermatozoa reaches the site of fertilisation with remarkable precision and timing. Several mechanisms have been proposed to guide sperm transport, including chemotaxis (movement along chemical gradients), rheotaxis (orientation against fluid flow) and thermotaxis (movement along temperature gradients) (Bahat and Eisenbach, 2006; Xiao et al., 2022; Eisenbach, 2025).

Among these, thermotaxis has attracted increasing attention as a potential mechanism involved in sperm navigation within the female reproductive tract (Xiao et al., 2022). Growing evidence indicates that thermotaxis plays an important role in sperm guidance. It was first reported in human and rabbit spermatozoa, and *in vitro* models showed that capacitated sperm responded to temperature gradients by adjusting their swimming direction (Bahat et al., 2003; Bahat et al., 2012; Boryshpolets et al., 2015). Human spermatozoa show a high degree of thermotactic sensitivity. Eisenbach (2025) has reported that they can respond to temperature gradients as small as 0.010–0.014 °C/mm, corresponding to the detection of temperature differences of less than 0.0006 °C. Some studies have also demonstrated that thermostatically selected spermatozoa display higher motility, membrane functionality, and fertilising capacity. These findings suggest that thermal responsiveness may be a biomarker of sperm quality (Pérez-Cerezales et al., 2018; Soto-Heras et al., 2023). Thermotaxis has been linked to the activity of thermosensitive ion channels. In particular, TRPV1 and TRPV4 (transient receptor potential vanilloid 1 and 4) act as intracellular sensors that enable sperm to detect small thermal cues (Pérez-Cerezales and Bermejo-Álvarez, 2017; De Toni et al., 2018; Mundt et al., 2018). New data indicate that the sperm-specific Ca^{2+} channel CatSper is directly activated by temperature at 33.5 °C, which explains the temperature-driven hyperactivation and provides a mechanistic bridge between hyperactivation, and flagellar movement (Swain et al., 2025). Further support for the physiological relevance of thermotaxis comes from studies conducted in several species (Bahat et al., 2005; Pérez-Cerezales et al., 2018; Ruiz-Diaz et al., 2023). Thermotactic responsiveness has also been associated with functional sperm quality, such as higher DNA integrity, motility and membrane stability, suggesting that thermotaxis may serve not only as navigational mechanism but also a means of sperm selection (Boryshpolets et al., 2015). In addition,

temperature alone can significantly influence sperm physiology. For example, elevated temperatures have been shown to induce hyperactivated motility in boar spermatozoa (Martin-Hidalgo et al., 2018). These findings suggest that thermotaxis may contribute both to sperm guidance and the regulation of sperm function. Despite numerous recent studies on sperm thermotaxis, its role in domestic livestock, particularly in rams, remains largely unknown. Reproductive success in ruminants depends on sperm motility, viability and structural integrity. Therefore, it is important to determine whether ram spermatozoa exhibit thermotactic behaviour. A better understanding of thermotaxis in ram spermatozoa may contribute to the development of improved sperm selection strategies for natural mating and assisted reproduction.

The objective of the present study was to assess the thermotactic behaviour of ram sperm under controlled *in vitro* conditions. A vertical thermotaxis model was established using 50 ml tubes divided into two compartments maintained at different temperatures (33 °C vs. 41 °C). After 60 min of incubation, sperm from each compartment was evaluated for concentration, motility, kinetic parameters, membrane function (HOST), and morphology.

Material and methods

The study was conducted at the Small Ruminant Research Unit of the Faculty of Agriculture, Cukurova University, Adana, Türkiye. All procedures involving animals were approved by the Animal Care Committee of Cukurova University (Protocol No. 12-6, 2024) and were conducted in accordance with the applicable ethical regulations.

Semen handling

Semen samples were obtained from one sexually mature Awassi ram (2 years old) maintained at the Small Ruminant Research Farm. Semen collections were performed once weekly during the autumn breeding season (September–November 2024). To minimise stress, the ram was placed in lateral recumbency, and the penis was gently exteriorised from the prepuce using a sterile gloved hand. Ejaculates were obtained by electroejaculation (EE) using a transrectal probe. Low-voltage and current pulses (2–3 V; ≤100 mA) were applied until ejaculation occurred. Semen samples were collected into a prewarmed sterile 50 ml plastic beaker and transported to the laboratory in an isolated container maintained at 38 °C for subsequent processing.

Sperm analysis

A detailed evaluation of each semen sample was conducted using the portable iSperm computer-assisted sperm analysis (CASA) system (Aidmics Biotechnology, Taiwan). This platform incorporates a high-resolution camera and dedicated software for the objective assessment of sperm quality and quantification of sperm kinetic parameters. Prior to analysis, semen samples were diluted 1:200 in an extender (OviPlus, Minitube, Germany) and maintained at 38 °C to simulate physiological conditions. Approximately 7–10 µl of diluted semen was loaded into a disposable microfluidic chip and sealed with a top cover to ensure even distribution. The chip was then inserted into the iSperm analyser (Aidmics Biotechnology, Taiwan), and measurements were performed using species-specific settings optimised for ram sperm (iSperm Ovine 6; Aidmics Biotechnology).

Sperm viability and membrane integrity assessment

Sperm viability was determined by nigrosine-eosin staining. A 10 µl aliquot of semen from each group was mixed with 10 µl of eosin and 20 µl of nigrosine stain on

a glass slide. After 30 s at room temperature, the mixture was spread as a thin smear on a second glass slide and allowed to air dry. Microscopic evaluation was performed under ×400 magnification using a phase contrast microscope (Leica DMR, Wetzlar, Germany), and at least 100 spermatozoa were evaluated per slide. Sperms with stained (pink/red) heads were classified as non-viable, whereas those with unstained (white) heads were classified as viable (Figure 1A).

Plasma membrane functionality was assessed using the hypo-osmotic swelling test (HOST). A 100 µl semen sample was mixed with 900 µl of a pre-prepared solution (100 mOsmol fructose), as described by Söderquist et al. (1997), and incubated at +37 °C for 30 min. Subsequently, a 10 µl aliquot of the mixture was transferred on a slide and examined at ×400 magnification using a phase contrast microscope (Leica DMR, Wetzlar, Germany). A total of 100 spermatozoa were evaluated, and the proportion of spermatozoa showing tail curling and swelling was recorded as an indicator of proper membrane function and expressed as a percentage (Figure 1B).

Experimental setup and thermotaxis model design

A custom-designed thermotaxis model was developed to assess sperm responses to vertical temperature gradients (Figure 2). The model was based on modified 50 ml tubes; each divided into two equal compartments by a 2 mm thick Styrofoam barrier extending to the 5 ml mark (Figure 2A–B). The barrier reduced heat transfer between compartments and limited sperm movement across the midline. Distinct thermal environments were established using independently controlled copper heating and cooling plates mounted externally on the upper portion of each tube (above the 5 ml mark). Heating was provided by a low-voltage power adapter (Gauss, Balıkesir, Turkey), while cooling was provided by a thermoelectric Peltier Module (2 V, 49 W, 40 × 40 mm;

Multicomp Pro, Leeds, UK). One compartment was maintained at 33 °C and the other at 41 °C, creating a consistent vertical temperature gradient with an accuracy of approximately ±1 °C. Temperature was monitored using digital sensors placed within each compartment and connected to a thermostat controller (STC-3008, ROC). The entire system was housed in a humidified incubator maintained at 38 °C 5% CO_2 throughout the 60 min incubation period (Figure 2C).

Semen sample preparation and incubation

Freshly collected semen samples with a minimum viability of 75% were included in the study. Isothermal control and vertical thermal gradient tubes (for thermotaxis) were filled with 20 ml of Sperm-TL solution (Caisson Lab., Utah, USA) supplemented with 3 mg/ml bovine serum albumin (BSA Frac. V, Sigma, Darmstadt, Germany) and 0.5 mM pyruvate, and pre-incubated for 2 h. A 100 µl aliquot of semen was carefully placed at the bottom of each tube using a pipette. Control tubes were maintained under isothermal conditions at 38 °C, whereas thermal gradient tubes were exposed to a temperature gradient of 33–41 °C, as described above. Sperm migration was assessed using the swim-up technique. The distribution of motile and live spermatozoa in thermal gradient zones was subsequently evaluated to determine thermotactic behaviour.

Sample collection and analysis

At the end of the 60 min incubation period, 1000 µl of sperm suspension was collected from each compartment in successive 100 µl aliquots to minimise disturbance of the thermal interface and prevent cross-contamination between compartments (Figure 2A). The collected samples were used for the assessment of sperm concentration and kinetic parameters using the iSperm computer-assisted sperm analysis (CASA)

system, sperm morphology by eosin-nigrosin staining, and membrane integrity evaluation using the HOST.

Statistical analysis

All statistical analyses were performed using Minitab 19. To reduce baseline variability, only ejaculates with ≥75% total motility was included in the study. The primary comparisons were between the 33 °C and 41 °C compartments (vertical gradient) and between the two compartments of the isothermal control (38a and 38b). Sperm concentrations, kinetic and quality parameters were evaluated in fresh semen (0 min) and after 60 min of incubation under the different thermal conditions (38 °C, 33 °C, and 41 °C). Paired comparisons were conducted between post-incubation measurements according to the experimental design. Paired tests were used to determine the differences between groups based on the experimental design. Variables with normally distributed differences were analysed using a paired *t*-test, while non-normally distributed variables were evaluated using the Wilcoxon signed-rank test. All results are reported as mean ± standard error of the mean (SEM).

Results

Sperm concentration

The effects of the vertical thermal gradient on ram sperm concentrations in the two compartments are presented in Figures 3 and 4. In the isothermal control, sperm were distributed evenly between the two compartments, with no significant difference in sperm concentration between them (both maintained at 38 °C). Mean (± SEM) sperm

concentrations were 12.7 ± 3.42 × 10^6/ml and 13.3 ± 3.53 × 10^6/ml in the two isothermal compartments, respectively (n = 7; Figure 3).

In contrast, under the 33–41 °C thermal gradient (Figure 4), mean (± SEM) sperm concentration was significantly higher on the cooler side (12.07 ± 1.55 × 10^6/ml) than on the warmer side (7.62 ± 1.30 × 10^6/ml; n = 16). Paired analysis confirmed a significant within-ejaculate difference (t (15) = 5.64, P < 0.001). Only one of the 16 paired observations showed a higher sperm concentration on the warm side, whereas all remaining pairs favoured the cool side. These results indicate temperature-dependent preferential sperm accumulation in cooler regions under the present steep thermal-gradient conditions

Sperm kinetics

Table 1 presents the effects of incubation time on sperm kinetic parameters in Awassi rams. Incubation time significantly affected sperm motility over the 60 min observation period at all temperatures. Total motility declined significantly from 83.9% in fresh semen to 67.3%, 62.8%, and 56.1% after 60 min at 38 °C, 33 °C, and 41 °C, respectively (P < 0.001). A similar trend was observed for progressive motility, which was highest in fresh samples (74.1 ± 4.0%) and lowest after incubation at 41 °C (50.6%, P < 0.001). Collectively, these results indicate a time dependent decline in total and progressive motility during the 60 min incubation period. In contrast, sperm velocity parameters, including curvilinear velocity (VCL), average path velocity (VAP), and straight-line velocity (VSL), were not significantly affected by incubation time (P > 0.05). Likewise, trajectory-related parameters, including linearity (LIN), straightness (STR), beat cross frequency (BCF), and wobble (WOB), remained unchanged (P > 0.05).

Amplitude of lateral head displacement (ALH) was the only trajectory parameter significantly affected by incubation time ($P < 0.002$).

Table 2 presents the effects of isothermal control conditions (38 °C, 60 min) on sperm kinetic parameters in Awassi rams. No significant differences were observed between the two compartments (38a and 38b) of the isothermal control for any of the evaluated parameters (n = 8; all parameters, $P > 0.05$). Accordingly, the mean (± SEM) values were similar in both compartments.

Table 3 presents the effects of a vertical thermal gradient (33 °C vs. 41 °C, 60 min) on sperm kinetic parameters in the Awassi rams. Following exposure of sperm from the same ejaculate to the thermal gradient, most kinetic parameters were comparable between the 33 °C and 41 °C compartments (n = 16). However, total motility was higher at 33 °C (62.4 ± 5.22%) than at 41 °C (56.4 ± 5.40%), approaching statistical significance ($P = 0.054$). Curvilinear velocity (VCL) also tended to be higher at 33 °C (163.5 ± 9.84 vs. 148.3 ± 12.20 μm/s; $P = 0.09$). Similarly, ALH showed an increasing trend at 33 °C (9.2 ± 0.34 vs. 7.70 ± 0.47 μm; $P = 0.051$). Overall, exposure to 41 °C was associated with modest reductions in motility and selected velocity-related parameters. However, most differences between temperatures were not statistically significant.

Sperm morphology

The HOST, performed to assess membrane integrity, showed a significant decline in the proportion of membrane intact sperm during incubation, regardless of the presence of a thermal gradient (Table 4). Under isothermal conditions (38 °C), the proportion of HOST-positive cells decreased significantly after 60 min of incubation compared with fresh semen ($P < 0.001$), indicating a loss of membrane function. A similar pattern was observed for sperm viability assessed by nigrosine-eosin staining (Table 4). Sperm

viability declined from 83.7% in fresh semen to 66.8% after 60 min under isothermal conditions and to 50.9% after incubation at 41 °C ($P < 0.001$). Thus, even in the absence of a thermal gradient, incubation time was associated with reductions in both sperm viability and membrane integrity.

The proportions of HOST positive and viable sperm were similar between the two compartments of the isothermal control, with no significant differences observed in either membrane integrity or viability (Table 5).

In contrast, under the thermal gradient, the proportion of HOST-positive sperm was higher at 33 °C (63.3%) than at 41 °C (44.5%) (Table 6). A similar result was obtained for sperm viability. After 60 min of incubation, the proportion of viable sperm decreased to 63.2% at 33 °C, and 50.9% at 41 °C. Both membrane integrity and sperm viability were significantly lower at 41 °C than at 33 °C ($P < 0.001$).

Discussion

This study provides experimental evidence that ram sperm can respond to temperature gradients in a thermotactic manner under controlled *in vitro* conditions. The results showed a clear within-ejaculate accumulation of sperm on the cooler side of a steep vertical gradient (33 °C vs. 41 °C), whereas no positional differences were observed under isothermal conditions (38 °C). Specifically, sperm concentration was significantly higher in the 33 °C compartment (12.07 million/ml) than in the 41 °C compartment (7.62×10^6/ml), indicating preferential migration towards the cooler side of the vertical gradient during swim up. This finding contrasts with the classical concept of sperm thermotaxis, in which capacitated spermatozoa migrate towards slightly warmer

regions along a temperature gradient. Such behaviour has been demonstrated in humans, rabbits, and mice, where sperm actively orient their movement towards higher temperatures by modulating their swimming pattern (Bahat et al., 2003; Bahat et al., 2012; Boryshpolets et al., 2015; Xiao et al., 2022). In the present model, which employed a steep temperature gradient (33 °C vs. 41 °C), sperm accumulated in the cooler region. This observation suggests that the direction of sperm migration may depend on both the magnitude of the gradient and the absolute temperatures involved. It is important to determine whether the observed response reflects true thermotaxis or avoidance of thermal stress at 41 °C. Although passive avoidance of heat-induced stress may have contributed to the observed distribution, several aspects of the experimental design suggest the involvement of an active behavioural response. First, a physical midline barrier (Styrofoam partition) separated the two compartments and prevented immediate fluid mixing, allowing sperm to distribute independently within the temperature gradient. Second, sperm were placed centrally, permitting free migration towards either compartment. Third, sperm were not immediately exposed to 41 °C but were free to migrate and accumulate in the region that provided more favourable conditions. Finally, both sperm motility and viability were preserved at 33 °C but were significantly reduced at 41 °C, suggesting that 41 °C likely imposed thermal stress on ram spermatozoa. This thermal stress may have contributed to the reduced sperm recovery from the warmer compartment by impairing motility and viability. In contrast, the *in vitro* environment in the 33 °C compartment may provide more favourable conditions for sperm (such as in the testis). Consequently, the observed accumulation at 33 °C can be interpreted as thermotactically driven movement towards a more favourable thermal environment. Similar heat sensitivity of sperm has been reported in other species, where sperm viability is compromised at temperatures significantly above the physiological

range (Martin-Hidalgo et al., 2018; Ruiz-Diaz et al., 2023). *In vivo*, temperature differences in the female reproductive tract are much more subtle; for instance, periovulatory gradients of 1–2 °C have been reported to guide sperm towards the site of fertilization in the rabbit oviduct (Bahat et al., 2005). Although the experimental setup used in the present study employed a relatively steep temperature gradient (33 °C vs. 41 °C), previous studies have shown that even extremely shallow gradients can induce thermotactic responses in human sperm (Bahat et al., 2012). These findings indicate that ram sperm are sensitive to thermal cues, consistent with reports of sperm thermotaxis in other mammals, including rabbits, mice, humans, horses and cattle (Roy et al., 2020).

The kinetic data showed that incubation at 38 °C for 60 min significantly reduced total and progressive motility, whereas most velocity and trajectory parameters (VCL, VAP, VSL, LIN, STR, WOB, BCF and ALH) were stable. These findings indicate that prolonged incubation primarily affected the proportion of motile sperm rather than the movement characteristics of the remaining motile cells. Similarly, exposure to a steep vertical temperature gradient resulted only in minor changes of most kinetic parameters between 33 °C and 41 °C. Nevertheless, total motility tended to be lower at 41 °C (P = 0.054), while VCL (P = 0.09) and ALH (P = 0.051) also showed a tendency to decrease. ALH, which reflects the amplitude of lateral head movement during sperm progression, is known to be sensitive to environmental and oxidative stress (Tanga et al., 2021). The reduction in ALH observed at 41 °C therefore supports the notion that elevated temperature may adversely affect sperm function, even when most kinetic parameters remain largely unchanged. ALH may also be associated with sperm hyperactivation, a process believed to facilitate thermotactic navigation (Boryshpolets et al., 2015). The reduction in progressive motility observed at 41 °C suggests that elevated temperature impairs forward sperm movement and disrupts flagellar function, consistent with

previous reports linking heat stress to alterations in flagellar activity and Ca^{2+} signalling (Martin-Hidalgo et al., 2018). Temperature-induced hyperactivation is a well-established phenomenon mediated by calcium influx through the CatSper channel (Mundt et al., 2018). Indeed, a recent study has demonstrated that CatSper, the primary sperm Ca^{2+} channel in sperm, is activated at temperatures above 33–34 °C and functions as a molecular thermosensor, triggering Ca^{2+} influx and downstream processes, including hyperactivation. This mechanism is instrumental in ensuring that sperm reach peak activity upon entering the warmer female reproductive track. However, temperatures exceeding the physiological range (>40 °C) may have detrimental effects on sperm function. The present study showed that sperm function was maintained in the moderate temperature range (33 °C), whereas motility and viability were significantly reduced at high temperature (41 °C).

The results showed that sperm viability and membrane integrity were influenced by temperature conditions during the 60 min incubation period. A time-dependent decline in the proportion of membrane intact and viable sperm was observed even under isothermal conditions at 38 °C. Moreover, nigrosine-eosin staining indicated a similar decline in sperm viability at 41 °C compared to 33 °C. Both viability and membrane integrity were lower at 41 °C than at 33 °C, consistent with the well-documented susceptibility of sperm membranes and metabolic pathways to heat-induced damage (Martin-Hidalgo et al., 2018; Swain et al., 2025). It has been suggested that thermotactically responsive sperm represent a higher-quality subpopulation, supporting the present findings showing greater viability and membrane stability at 33 °C (Xiao et al., 2022). Previous studies on ram sperm have similarly demonstrated their susceptibility to thermal damage, reinforcing the importance of temperature optimisation in small ruminants (Söderquist et al., 1997). This result aligns with the established view

that sperm are highly sensitive to heat stress. Even moderate increases in temperature above the physiological range may compromise membrane integrity, activate apoptotic pathways, and induce protein denaturation, ultimately resulting in cell death (Martin-Hidalgo et al., 2018; Ruiz-Diaz et al., 2023). At the molecular level, sperm thermotaxis is believed to involve transient receptor potential vanilloid (TRPV) channels, particularly TRPV1 and TRPV4 (De Toni et al., 2018; Mundt et al., 2018). These thermosensitive channels regulate calcium influx in response to temperature changes, influencing sperm flagellar activity. Recent studies have also indicated a role of opsins, including rhodopsin and melanopsin, which may function as thermal sensors in sperm cells (Roy et al., 2020). In addition, the main sperm specific CatSper channel is directly gated by temperature and becomes activated at approximately 33–34 °C, which is consistent with the thermal sensitivity of ram sperm demonstrated in the present study (Swain et al., 2025).

The presence of thermotaxis in ram sperm suggests that temperature gradients may play a role in guiding sperm within the female reproductive tract of small ruminants. To date, comparable data for sheep and goats are lacking. The current study demonstrated that ram sperm are capable of thermotactic orientation; however, further research is required to determine whether physiological temperature gradients exists in the ewe oviduct around the time of ovulation, as has been reported in rabbits. There is a growing interest in developing sperm selection techniques that mimic natural selection mechanisms within the female reproductive tract to improve the quality of sperm used for IVF (Ruiz-Diaz et al., 2023). Microfluidic devices have been developed to utilise temperature gradients for the isolation of motile and thermotactically responsive sperm subpopulations (Huang et al., 2024). Additionally, sperm selected under thermal gradients have been associated with improved fertilisation outcomes in animal models

(Pérez-Cerezales et al., 2018; Ruiz-Diaz et al., 2023). The findings of the present study suggest that a similar strategy may be applicable to small ruminants.

Conclusions

In conclusion, the present study demonstrates that ram spermatozoa are capable of thermotactic responses to a temperature gradient, accumulating in a more favourable thermal environment while maintaining motility and viability. The findings also show that elevated temperatures compromise sperm motility and survival, underscoring the importance of temperature control during sperm handling and assisted reproductive procedures even under the non-physiological gradients used in this study to elucidate underlying mechanisms. Further investigations into this phenomenon may improve our understanding of the processes that enable sperm to successfully reach and fertilise the oocyte.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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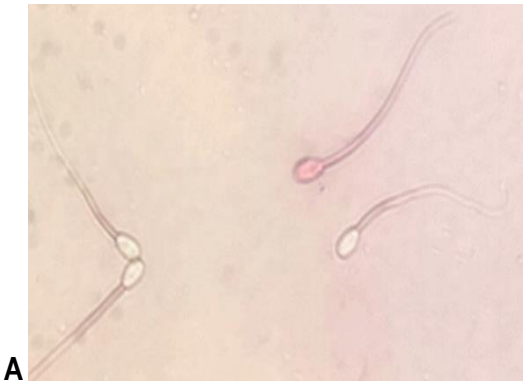

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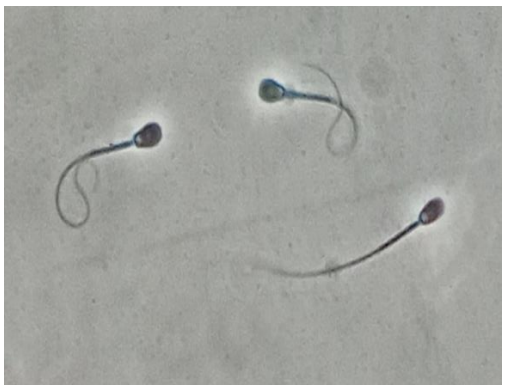

B

Figure 1. (A) Nigrosine–eosin staining used to assess viability (dead = pink/red heads; live = unstained). (B) Hypo-osmotic swelling test (HOST) for membrane integrity; tail curling indicates intact membrane

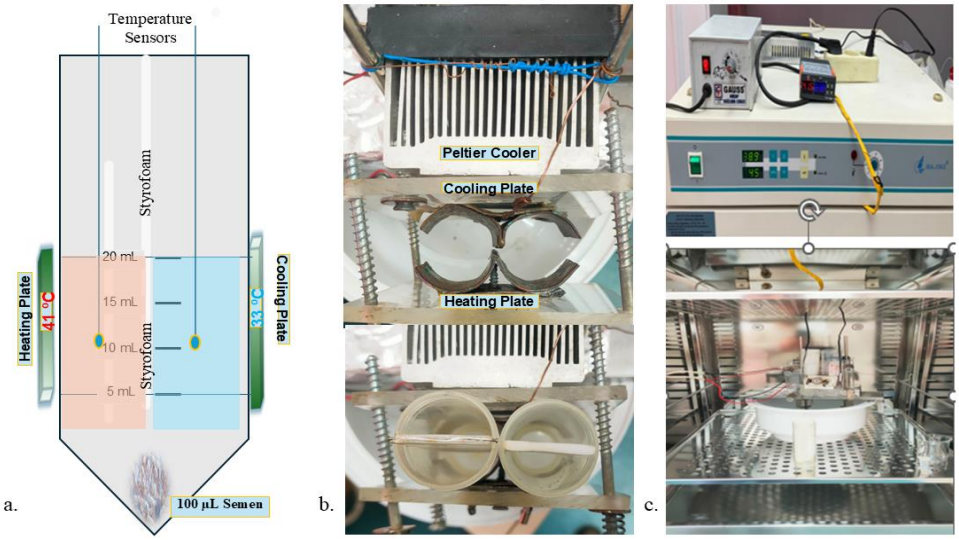

Figure 2. Custom vertical thermotaxis setup showing (A) modified 50 ml tube with Styrofoam midline barrier, (B) external copper plates for heating/cooling, (C) CO_2 incubator set up of the system

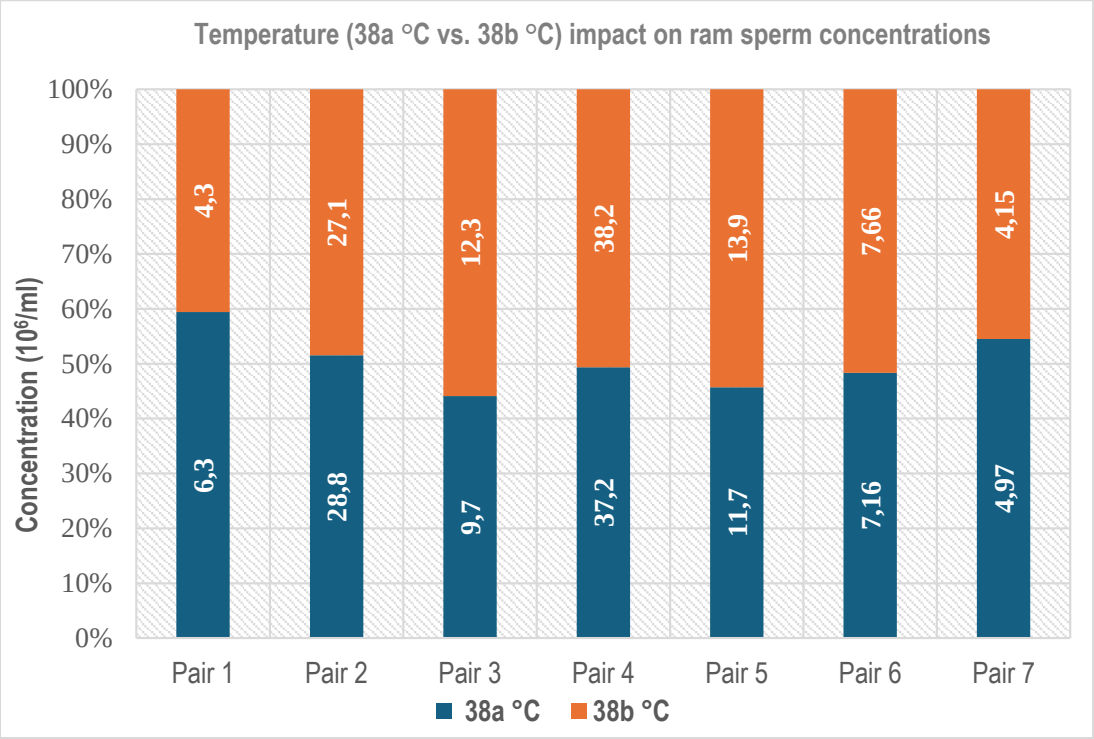

Figure 3. Isothermal gradient (38a°C vs. 38b°C) effects on ram sperm concentration

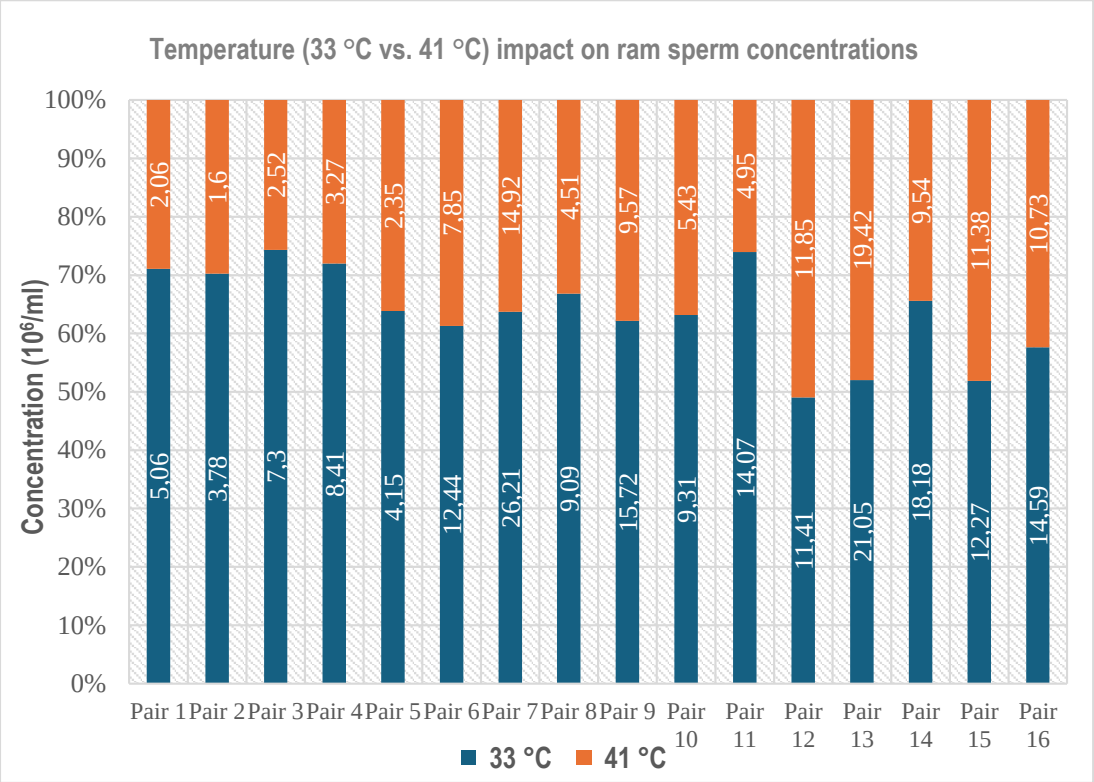

Figure 4. Vertical thermal gradient (33 °C vs. 41 °C) effects on ram sperm concentration

Table 1. Time (fresh (0 min) vs. 60 min) effect on the kinetic features of ram sperm

Time/Kinetics	Fresh (0 min) n = 7	38 °C (60 min) n = 16	33 °C (60 min) n = 32	41 °C (60 min) n = 32	*P*-value
Motility, %	83.9 ± 3.94[a]	67.3 ± 4.90[b]	62.8 ± 5.20[b]	56.1 ± 5.42[b]	0.001
Progress. motility, %	74.1 ± 4.07[a]	55.9 ± 3.76[b]	56.3 ± 4.76[b]	50.6 ± 5.21[b]	0.001
Curvilinear velocity, µm/s	160 ± 5.82	149 ± 4.94	164 ± 9.74	147 ± 12.2	NS
Average path velocity, µm/s	98.1 ± 7.42	90.8 ± 5.32	99.9 ± 10.1	91.3 ± 9.54	NS
Straight line velocity, µm/s	87.3 ± 7.35	85.8 ± 1.64	82.6 ± 3.25	81.6 ± 5.07	NS
Linearity, %	54.3 ± 4.82	50.7 ± 1.56	53.4 ± 4.48	52.6 ± 4.57	NS
Straightness, %	86.1 ± 2.35	85.8 ± 1.64	82.6 ± 3.25	81.6 ± 5.07	NS
Amplitude of lateral head displacement, µm	9.8 ± 0.33[a]	9.02 ± 0.66[b]	9.14 ± 0.35[b]	7.81 ± 0.48[b]	0.002
Beat cross frequency, Hz	28.6 ± 1.70	31.4 ± 1.63	32.2 ± 1.52	30.7 ± 1.97	NS
Wobble, %	61.3 ± 4.62	58.5 ± 1.66	60.4 ± 3.92	57.1 ± 4.33	NS

data are presented as mean value ± SEM; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 2. Effect of temperature on ram sperm kinetic features in the isothermal group

Temperature/Kinetics	38a °C (60 Min) n = 8	38b °C (60 Min) n = 8	*P*-value
Motility, %	69.1 ± 4.95	65.5 ± 8.80	NS
Progressive motility, %	57.2 ± 2.53	54.5 ± 7.34	NS
Curvilinear velocity, µm/s	150.0 ± 6.22	148.6 ± 8.50	NS
Average path velocity, µm/s	83.9 ± 4.18	97.6 ± 9.92	NS
Straight line velocity, µm/s	77.1 ± 5.65	76.7 ± 7.27	NS
Linearity, %	50.6 ± 2.28	50.8 ± 2.30	NS
Straightness, %	85.4 ± 1.68	86.3 ± 2.94	NS
Amplitude of lateral head displacement, µm	9.7 ± 0.73	8.4 ± 1.10	NS
Beat cross frequency, Hz	30.4 ± 1.75	31.84 ± 2.86	NS
Wobble, %	60.1 ± 2.39	56.9 ± 2.32	NS

data are presented as mean value ± SEM; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 3. Effect of temperature on ram sperm kinetic features in the thermal gradient group (33 °C vs. 41 °C)

Temperature/Kinetics	33 °C (60 min) n = 16	41 °C (60 min) n = 16	*P*-value
Motility, %	62.4 ± 5.22	56.4 ± 5.40	0.054
Progressive motility, %	56.1 ± 4.78	50.8 ± 5.20	NS
Curvilinear velocity, µm/s	162.5 ± 9.84	148.3 ± 12.20	0.09
Average path velocity, µm/s	97.1 ± 9.91	94.2 ± 9.82	NS
Straight line velocity, µm/s	86.9 ± 10.10	86.7 ± 9.34	NS
Linearity, %	52.2 ± 4.32	53.2 ± 4.72	NS
Straightness, %	82.1 ± 3.22	82.0 ± 5.10	NS
Amplitude of lateral head displacement, µm	9.2 ± 0.34	7.7 ± 0.47	0.051
Beat cross frequency, Hz	31.9 ± 1.58	30.9 ± 1.94	NS
Wobble, %	59.2 ± 3.76	58.3 ± 4.51	NS

data are presented as mean value ± SEM; $P > 0.05$ (no statistically significant)

Table 4. Effect of time on live (%) and HOST positive (%) ram sperm in the thermal gradient groups

Time/Morphology	Fresh (0 Min) n = 7	38 °C (60 Min) n = 16	33 °C (60 Min) n = 32	41 °C (60 Min) n = 32	*P*-value
HOST positive, %	85.3 ± 3.94[a]	63.9 ± 2.49[b]	63.3 ± 1.86[b]	44.5 ± 1.98[b]	0.001
Live sperm, %	83.7 ± 4.07[a]	66.8 ± 3.96[b]	63.2 ± 3.09[b]	50.9 ± 2.98[b]	0.001

data are presented as mean value ± SEM; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 5. Effect of temperature on live (%) and HOST positive (%) ram sperm in the isothermal group (38a °C vs. 38b °C)

Temperature/Morphology	38a °C (60 min) n = 8	38b °C (60 min) n = 8	*P*-value
HOST positive sperm, %	67.1 ± 4.61	66.3 ± 3.85	NS
Live sperm, %	63.1 ± 2.20	64.8 ± 2.54	NS

data are presented as mean value ± SEM; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 6. Effect of temperature on Live (%) and HOST positive (%) ram sperm in the vertical thermal gradient group (33 °C vs. 41 °C)

Temperature/Morphology	33 °C (60 min) n = 16	41 °C (60 min) n = 16	*P*-value
HOST positive sperm, %	63.3 ± 1.86[a]	44.5 ± 1.98[b]	0.001
Live sperm, %	63.2 ± 3.09[a]	50.9 ± 2.98[b]	0.001

data are presented as mean value ± SEM; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$