

BASIC SCIENCE

Establishing a Thermal Imaging Technology (IRT) Based System for Evaluating Rat Erectile Function



Shuai Liu, MD, PhD,^{1,2,#} Zhendong Zhao, MD,^{1,2,#} Ziwen Wang, MD,¹ Tongxiang Diao, MD, PhD,¹ Keqin Zhang, MD, PhD,¹ Hui Zhang, MD, PhD,¹ Dingqi Sun, MD, PhD,¹ Feng Kong, MD, PhD,¹ and Qiang Fu, MD, PhD^{1,2}

ABSTRACT

Introduction: Measurement of intra-cavernous pressure (ICP) is an internationally recognized method to evaluate erectile function of animals, however, this process is invasive, destructive, and cannot be repeated, leading to a daunting challenge for monitoring the changes in erectile function throughout the whole treatment duration.

Aim: To verify whether infrared ray thermography technology based system could be a good substitution of ICP for evaluating rat erectile function.

Methods: A novel thermal image-based method, infrared ray thermography technology (IRT) was employed to monitor erectile function in erectile dysfunction (ED) rats. To detect the sensitivity and specificity of this new technology, 4 ED rat models (Diabetic, nerve-injury, vascular-injury and aged ED models) were established and subjected to both ICP and IRT test.

Outcomes: Statistical comparisons were done to test the effectiveness of this new way for detecting and dynamically monitoring erectile function.

Results: Based on the data curves obtained from ICP and IRT, the IRT showed a similar trend (including peak value, climbing speed) as that of ICP. IRT is considered as a precise way to monitor the real-time changes of erectile function in all ED rat models. The AUC of peak temperature detected by IRT in DMED, aged ED, vascular-injury ED, the nerve-injury ED and total ED rat models were 0.9811, 0.9836, 0.9893, 0.9989 and 0.9882, respectively. Meanwhile, the AUC of temperature climbing rate were 0.6486, 0.8357, 0.9184, 0.8675 and 0.8168. Also, it is a non-invasive process of dynamically monitoring erectile function of a same rat at different time points (before and after drug intervention). The data showed that the real-time recovery by tadalafil was obtained by IRT methods even after treatment for only 2 weeks in the diabetic ED (DMED) rat model.

Conclusion: A novel noninvasive method for monitoring erectile function in rat ED models was established, and can replace or supplement ICP test. **Liu S, Zhao Z, Wang Z et al. Establishing a Thermal Imaging Technology (IRT) Based System for Evaluating Rat Erectile Function. Sex Med 2022;10:100475.**

Copyright © 2021 The Authors. Published by Elsevier Inc. on behalf of the International Society for Sexual Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Key Words: Erectile Dysfunction; Infrared Ray Thermography Technology; Intra-Cavernous Pressure

Received March 2, 2021. Accepted November 9, 2021.

¹Department of Urology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, SD, China;

²Department of Urology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, SD, China

[#]Shuai Liu & Zhendong Zhao contribute equally

Copyright © 2021 The Authors. Published by Elsevier Inc. on behalf of the International Society for Sexual Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

<https://doi.org/10.1016/j.esxm.2021.100475>

INTRODUCTION

Erectile dysfunction (ED) is an inability to attain or maintain penile erection adequately, leading to dissatisfaction of sexual relationship.¹ Currently, the pathogenesis of ED is still unclear, as it might occur due to psychological, neurological, hormonal, arterial, or cavernosal impairment or from a combination of these factors.² From 1989, rats have become a predominant animal model for investigating erectile function.³

Intra-cavernous pressure (ICP) test is an internationally recognized method for evaluating ED in rats. ICP is measured by implanting a pressure transducer by connecting to the penile crura, and this sends signals through a telemetric device or by

tunneling a heparinized catheter from the penile crura to the pressure transducer.³⁻⁵ However, this is not considered as an optimal approach as manipulation and disruptive dissection causes injury to the structures that are essential for erectile function, limit the reproducibility and long-term observation of erectile state in rats after drug intervention.

In this study, a new laboratory technique for detecting erectile function in rats has been proposed. Infrared ray thermography technology (IRT) is non-contact, noninvasive and repeatable,⁶⁻⁸ IRT has been widely employed in detecting cancer metastasis as well as early diagnostic of other diseases. Thanks to advances in this technology, it allows to real-time monitor the temperature. The essence of a dilated penis is congestion, which brings blood pressure climbing as well as temperature rising, and that inspired us to make attempts to establish a new method to monitor hyperemia of the penis, which reflects erectile function by using IRT (2). However, we found there would be minor delay in temperature change compare to pressure change, thus, whether IRT can possess the capacity to reflect ICP test effectiveness precisely needs further study, besides, how well this method in standardized, stable practice of penile erectile function test? This study was designed to verify whether IRT is a promising way to replace or supplement ICP test in rat ED models. (1)

MATERIAL AND METHODS

Animals

All procedures in this study were approved by the Institutional Animal Care and Use Committee at Shandong University (NO.2020-1001). Eighty 12-week-old male Sprague-Dawley (SD) rats and 20 18-month-old male SD rats were obtained from the Animal Breeding Center at Shandong University.

ED is a human condition, in our study, 4 classic "ED" rats model were employed. As all we know, impaired penile tumescence is the core mechanism of human erectile dysfunction, in our study, although it is not accurate to define "impaired penile tumescence" as human "ED", at least in part, it would be seen as a simulation of human erectile dysfunction. The rats were divided into 5 groups including: a normal control group, a diabetes mellitus-induced (DMED) model group, a nerve-injury ED model group, a vascular-injury ED model group and an aged ED model group, with 20 rats per group ($n = 100$) (Figure 3).

After the models were successfully established, tadalafil was orally administered for 6 weeks (daily oral gavage at a dose of 5mg/kg, 10 rats per group) After 6 weeks, ICP and thermography were respectively analyzed on treated and untreated ED models to determine the improvements of erectile function.

Diabetes Mellitus Erectile Dysfunction (DMED) Model.

After an overnight fasting, 20 randomly selected SD rats were injected with a single intra-peritoneal injection of 60 mg/kg

streptozotocin (STZ, Sigma-Aldrich Chemical Co, St. Louis, MO). Next, blood samples were obtained (after 72 hour of STZ or vehicle injection) by pricking the tail for blood glucose measurement. Twenty STZ-treated rats had random blood glucose concentrations of greater than 16.67 mmol/L and diabetes was successfully induced in the rats. The blood glucose levels and body weight of all rats were monitored weekly for the first month, and then monthly thereafter.

Nerve-Injury ED Model. Twenty randomly selected SD rats were induced with 5% sodium pentobarbital anesthesia. A vertical midline incision was made till the lower abdomen. The major pelvic ganglia (MPG) and the CN were exposed behind the prostate, and then hemostat was applied with full tip closure to each CN 1 mm distal to the MPG for 2 minutes.

Vascular-Injury ED Model. Twenty randomly selected SD rats were induced with 5% sodium pentobarbital anesthesia. A vertical midline incision till the lower abdomen was made to expose and locate the common iliac artery. The microplastic soft catheter with a length of 2 mm was longitudinally cut with microsurgical scissors, and the soft catheter with the help of dissecting microscope was carefully wrapped in the separated internal iliac artery with microsurgical forceps, and then a soft catheter was fixed with No.6 silk thread. After operation, the rats were fed with high fat diet (Product #D12492, Beijing Keaoxili feed co., ltd, Beijing, China.) for 8 weeks post-surgery.

Aged ED Model. Rats of 20-months-old were used aged ED model, and >90% aged rats were confirmed as having erectile dysfunction.

Stimulation of Erection

2 Ways for Stimulating Rats' Erection Were Employed.

Cavernous nerve (CN) stimulation. To verify whether IRT had similar detection effect as ICP, traditional nerve stimulation were conducted. The cavernous nerve located just below the stellate ganglion was exposed and hooked up to electrodes. The following CN stimulation parameters were set on a stimulator PowerLab 26T (Ad Instruments, Sydney, Australia): a current was set at 1.5 mA, with a frequency of 16 Hz, and a voltage of 3 V with a pulse width of 5 ms. In addition, 50 sec of stimulation was applied between the stimuli with a rest period of at least 1 min.

For persistent monitoring of erectile function in a single animal, penile erection was stimulated by injecting apomorphine (APO) instead of electrical nerve stimulation. Penile erection was stimulated by subcutaneous injection of apomorphine (APO, 100 μ g / kg). Although it is not as accurate as CN stimulation, it can be repeated on a single animal.

IRT Measurement. The instruments used included an inhalation anesthesia device, a thermostatic rat table and a rectal temperature measuring device. The thermal imager FLIR T540 (FLIR systems, Boston, MA) equipment was assembled (Figure 2). The rats were housed in an operating room to allow them to adapt to the environment for at least 15 minutes. After anesthetizing, the rat was placed on a constant temperature platform. It is important to remove the hair without skin damaged to void significant bias. The thermal imager FLIR T540 (FLIR systems, Boston, MA) was present on the side of the rat and 25 cm away from the rat penis. Next, a thermometer was inserted into the rectum of the rat. After erectile stimulation by CN stimulation or APO subcutaneous injection, the erectile status of the rat was observed (Figures 1 and 2).

Instrument Operation of IRT Measurement. The parameters of thermal imager were adjusted, and the lens center of the thermal imager was aligned with rat penis. The thermal imager was present on the side of the rat and 25 cm away from the rat penis. Next, a thermometer was inserted into the rectum of the rat. The auto focus button of the thermal imager was used, and then the recording button was used. It is important to note that the experimenter was forbidden to walk around the laboratory when the recording was conducted.

Intra-Cavernous Pressure Measurement. The intra-cavernous pressure (ICP) and the ratio of ICP/mean systemic arterial pressure (MAP) were used to measure erectile function by electrical stimulation of CN. After CN was identified and isolated, IRT Measurement was conducted before ICP test, following closely, a 26-gauge needle filled with heparinized saline was inserted into the cavernosum to measure the ICP as stimulating CN. The left carotid artery of each rat was cannulated with a PE-50 tube (Intramedic; Becton Dickinson & Co., Sparks, MD) to facilitate continuous measurement of MAP after induction with 5% sodium pentobarbital anesthesia. Both pressure lines (ICP and MAP) were measured continuously with a pressure transducer system PowerLab 26T (AD Instruments, Sydney, Australia) (AD instruments, Australia) after CN stimulation. The data were collected and analyzed by using supporting software (labchart8 for Windows, AD instruments, Australia).

Statistical Analysis

The continuous variables are expressed as median with interquartile ranges (IQR). Correlations between the values were detected by IRT and the values detected by ICP were evaluated by Spearman correlation analysis. The area under the receiver operating characteristic curve (AUC) was used to assess on how

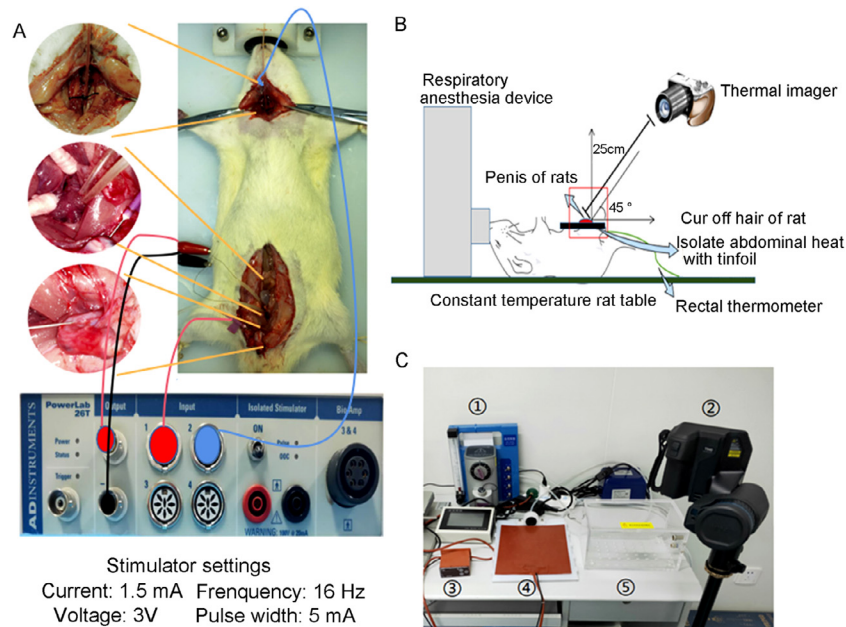


Figure 1. Schematic diagram of intra-cavernous pressure (ICP) test and infrared thermography technology (IRT) detection.

A: Schematic diagram of ICP detection. The erection of rat was stimulated by electrophysiological instrument MAP and ICP was measured after aorta intubation and penile intubation, causing irreversible damage (TNI012/ST Pulse Transducer, ML4856, ADINSTRUMENTS Australia). The CN stimulation parameters were shown.

B: Schematic diagram of IRT detection. The thermal imager was placed 25 cm away from the rat penis, and a thermometer was inserted into the rat rectum to normalize individual variation;

C: Instruments of IRT detection were shown. ①respiratory anesthesia machine pumps 3% isoflurane. ②thermal image ③rectal thermometer ④constant temperature rat platform and ⑤anesthesia induction box. A real-time display interface of IRT detection.

Acquired by thermometer showing temperature focus based on rat penis, ranging from blue (flaccid condition) to red (erectile condition).

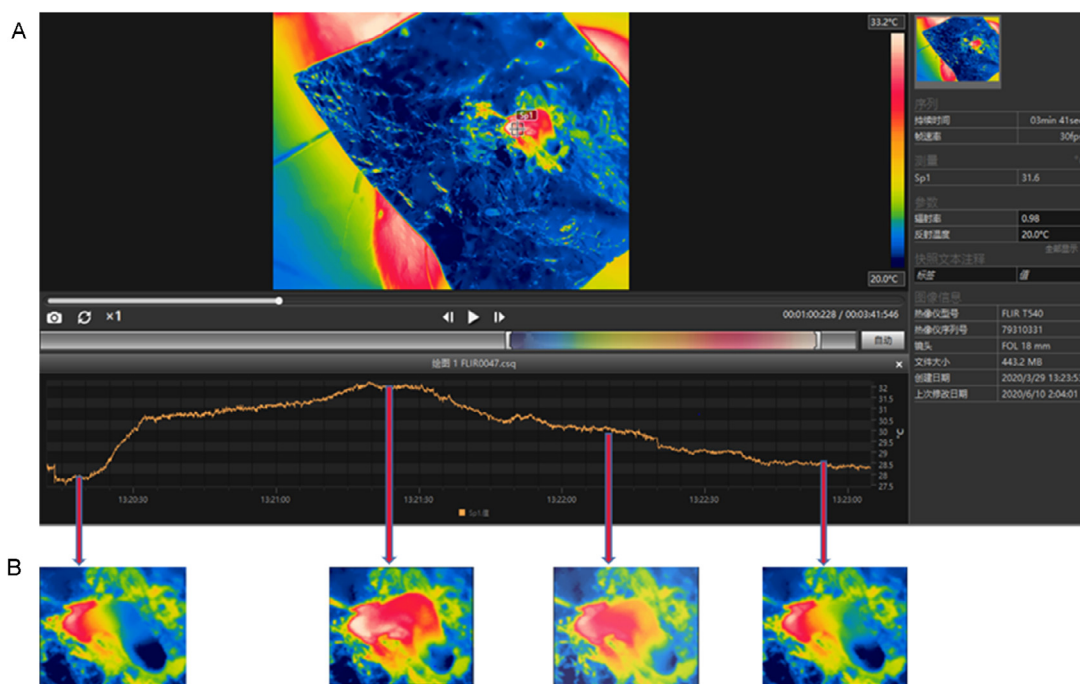


Figure 2. Automatic real-time curve was drawn according to the local penis temperature (automatic focusing).

A: This operation interface showed dynamic monitoring of erectile procedures based on IRT test. Automatic focus were set to tracing a fixed location on penis, and it could be correct in data analysis procedure by using supporting software (FLIR Tools+ 5.7, Sweden). B: A whole procedure of erectile was recorded, color from blue to red indicating a temperature climbing during this procedure; After the stimulation was removed, the color of focus area turned to blue, indicating a temperature decline. Changes in temperature and erection angle are synchronized in real time indicating a close relation between temperature and blood supply during procedure of erectile. More details can be found in supplementary data (video.wmv).

well the values of IRT discriminated ED in rats. All data were analyzed using R software version 3.6.3 (<https://www.R-project.org/>), and the pROC package were used to acquire the ROC curve, sensitivity, specificity, NPV, and PPV of different groups.

RESULTS

Data From IRT Test Showed Similar Detection Efficiency as Compared With ICP Test

The data fitting curves from IRT showed similar erectile function curve trends as compared to ICP test (Figure 4). ICP was measured under anesthesia after IRT in the same rat, and found that the curve obtained by IRT measurement was similar to that of ICP. The temperature reached to a peak value when starting the stimulation and the temperature gradually returned to baseline after removing the stimulation (Figure 4). Similar curve graph was obtained according to the data gained from IRT and ICP, indicating that IRT exhibit the same efficiency as ICP.

To further verify this hypothesis, 4 classic ED rat models were established. Both ICP and IRT were conducted on these 4 ED rat models to test the sensitivity and specificity of IRT when compared to ICP. Also the erectile function improvement were distinguished by both ICP and IRT after tadalafil treatment (ED group vs treatment group) to show whether the IRT test can distinguish the improvement of erectile function in these 4 rat ED

models. The data of ICP and IRT of diabetes mellitus-induced erectile dysfunction (DMED) model, aged ED model, vascular-injury ED model and the nerve-injury ED model was shown in the following figures (Figure 4), respectively.

After treatment, the ICP and IRT images of the above models were obtained in the same way. The peak value of ICP curve was found to be decreased in sick rats, and the peak value of IRT curve was also decreased, showing similar trends in both. After treatment, the peak values of both ICP and IRT curves were found to be increased. Moreover, each model has its own characteristics. After applying the stimulus to the DMED model, the ICP could reach its peak quite promptly; however, after reaching its peak, the pressure was soon shown to be spontaneously decreased, and could not last for a prolonged period of time (Figure 4).

After stimulating the nerve-injury in ED model, a peak was achieved in ICP for a prolonged period of time. The vascular-injury ED model and the senile model displayed comprehensive characteristics of DMED and nerve-injury ED models. These results demonstrated that the data from IRT test had similar detection efficiency as that of the ICP test, and the data fitting curve from IRT showed the same erectile function curve trends as did ICP. These results demonstrated that the data from IRT test had similar detection efficiency as that of the ICP test, and the data fitting curve from IRT showed the same erectile function curve trends as did ICP.

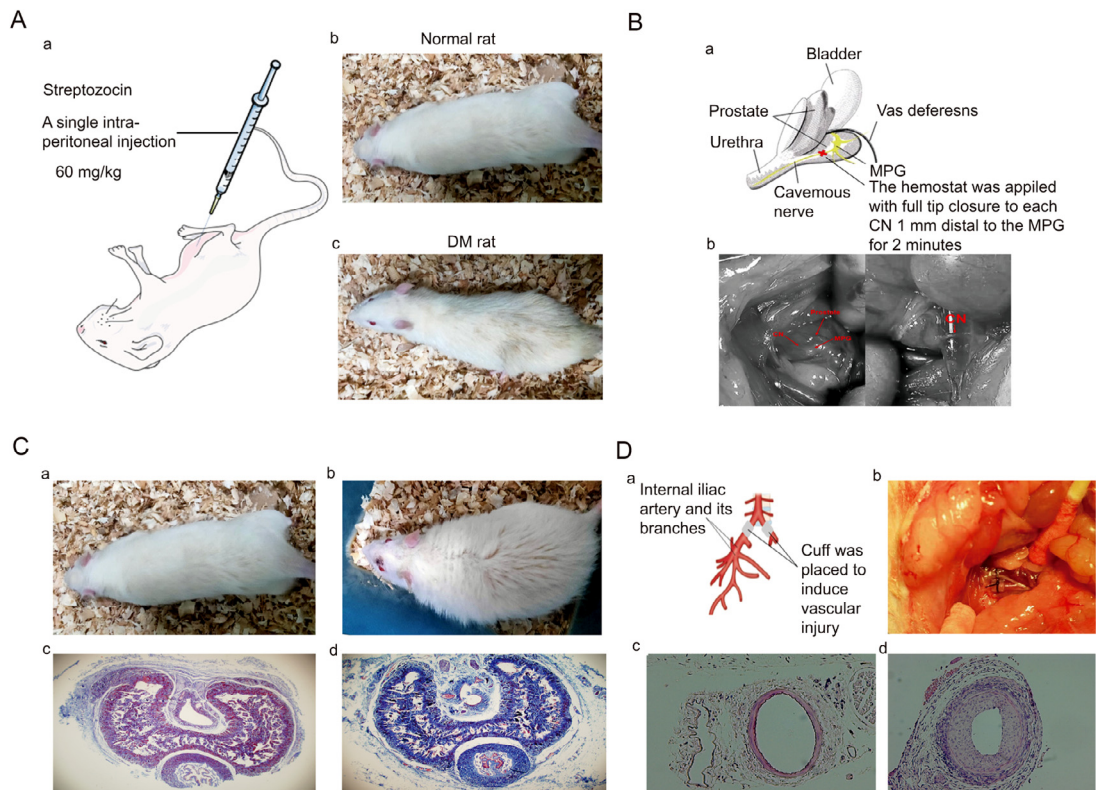


Figure 3. The establishment of 4 classic erectile dysfunction rat models.

A. The establishment of DMED rat model was shown. a: Schematic diagram of establishment of diabetic rat model. After 8 weeks, >95% rats were confirmed suffering from erectile dysfunction; b: normal control rats; c: DM rats. Diabetic rats are more emaciated, with yellowish hair of no luster.

B. The establishment of Nerve-injury ED rat model was shown. A: Schematic diagram of establishing a nerve injury model. The hemostat was applied with full tip closure to each CN 1 mm distal to the MPG for 2 minutes; >95% rats were confirmed with erectile dysfunction; b: The localization of cavernous nerve and MPG.

C. The establishment of aged ED rat model. a: normal rat; b: aged rat. The aged rats were more obese with thin hair; c, d: Masson trichrome staining of penile midshaft specimens, and comparison to normal control rats (c), less actin and more collagen were confirmed in aged rats (d). >90% aged rats were confirmed with erectile dysfunction;

D. The establishment of vascular-injury ED rat model. a: Schematic diagram of establishing vascular injury models. The microplastic soft catheter with a length of 2 mm was cut longitudinally with microsurgical scissors; the soft catheter (cuff) was carefully wrapped in the separated internal iliac artery with microsurgical forceps under the dissecting microscope, and then the fixed with a silk thread. After operation, the rats were fed with high-fat diet for 8 weeks post-surgery, >95% rats were confirmed with erectile dysfunction; b: operation of cuff placement; c, d: HE staining of internal iliac artery of normal rats (c) and vascular-injury rats (d). The intima of vessels with cuff was thickened and the lumen became narrow.

To verify the sensitivity and specificity of IRT test, statistical analysis has been done. Correlation analysis implicated striking coincidence between IRT and ICP (Figure 4). Figure 5 showed peak temperature and temperature climbing rate of IRT ROC curve in 4 rat models and the area under the ROC curve (AUC) was used to assess on how well the values of IRT discriminated the ED in the 4 rat models. AUC of peak temperature detected by IRT in DMED, aged ED, vascular-injury ED, the nerve-injury ED and total ED rat models were 0.9811, 0.9836, 0.9893, 0.9989 and 0.9882, respectively. AUC of temperature climbing rate as detected by IRT in DMED, aged ED, vascular-injury ED, the nerve-injury ED and total ED rat models was 0.6486, 0.8357, 0.9184, 0.8675 and 0.8168. Correlations between IRT and ICP were evaluated by spearman correlation analysis.

Results showed that the peak temperature and temperature climbing rate as detected by IRT showed positive correlation with peak pressure and pressure climbing rate by ICP (Figure 5 and Table 1). According to the ROC curve, a detailed cutoff value of sensitivity, specificity, NPV and PPV of peak temperature and temperature climbing rate by IRT in different models.

IRT Test Enables Persistent Monitoring of Erectile Function in a Single Animal

As described above, the major deficiency of ICP was the penile destruction and invasion caused by piezometer tube puncturing, brings a challenge to monitor the changes of erectile function throughout the whole treatment duration.

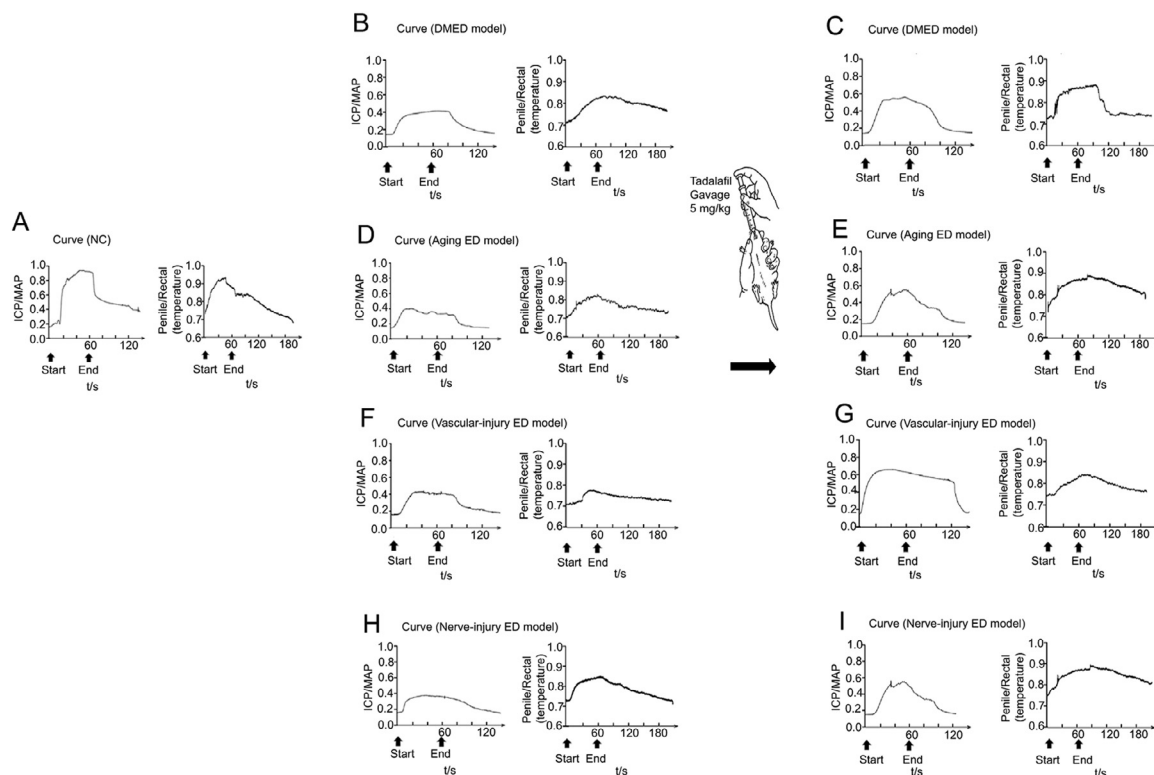


Figure 4. Comparison of curve graph of IRT and ICP based on real-time data gained from Normal control and 4 ED models.

The erectile procedures were stated by CN stimulation as described above; the ICP/MAP data were obtained by electrophysiological instrument; the temperature was monitored by thermal imager, a thermometer was inserted into the rectum of the rats to normalize individual variation. After removing the stimulation, the ICP and temperature were gradually returned to baseline. A, Normal control rats; B,C: DMED models; D,E: Aged ED models; F,G: vascular-injury ED models; H,I: nerve-injury ED models.

To monitor erectile function of the single diabetes mellitus (DM) rat for a prolonged period of time, penile erection was stimulated by injecting apomorphine instead of electrical nerve stimulation. Thermal imaging was then used to monitor the erection process at 4 time points: before DMED model establishment, after DMED model establishment, as well as 2 and 6 weeks after tadalafil treatment (5mg/kg daily oral gavage, Figure 6). The results revealed that IRT clearly showed erectile function of each rat at different periods and states. The images showed that the peak temperature (Peak T) of diabetes mellitus (DM) rats at week 6 after treatment was clearly higher than that of untreated rats, indicating better blood supply after treatment, the improvement of erectile function can be distinguished even after 2 weeks. This indicated that IRT could monitor erectile function of rats and make possible long-term dynamic monitoring of erectile function of rats.

DISCUSSION

Thermal imaging technology involves the use of a thermal imager to receive infrared radiation energy, transforming the invisible infrared radiation into a visible image. Hitherto, the sensitivity and specificity of thermal imaging technology for detecting breast cancer are as high as 90 percent.⁹ Thermal imaging technology has increasingly become more popular in clinical

practice. It does not require contact, non-invasive and enjoys long-term dynamic monitoring characteristics.⁶⁻⁸ However, No similar reports as thermal imaging technology was used in erectile function monitoring on lab rats have been seen so far

Today, rigiscan test were widely used in erectile dysfunction as well as curative effect evaluation in human, however, there is still lack of methods in lab animal erectile function evaluation without invasive, brings the challenge in long-term dynamic monitoring and drug effect evaluation in laboratory practice.

Penile erection is a coherent and orderly process, intracavernous pressure (ICP) test is an internationally recognized method for evaluating ED in rats, and it is qualitative methodology to show erectile function indirectly by presenting blood pressure, during this procedure, not only the highest pressure, but also the velocity of pressure change are recorded. In this study, temperature trend were employed to be an alternative of pressure change, it is not perfect, but it might be a potential alternative.

In our practice, we found although data from IRT test showed similar detection efficiency as compared with ICP test, there are still significant flaws caused by delayed effect in temperature changing. In our further study, the linear relationship between pressure and temperature will be analysed, algorithm

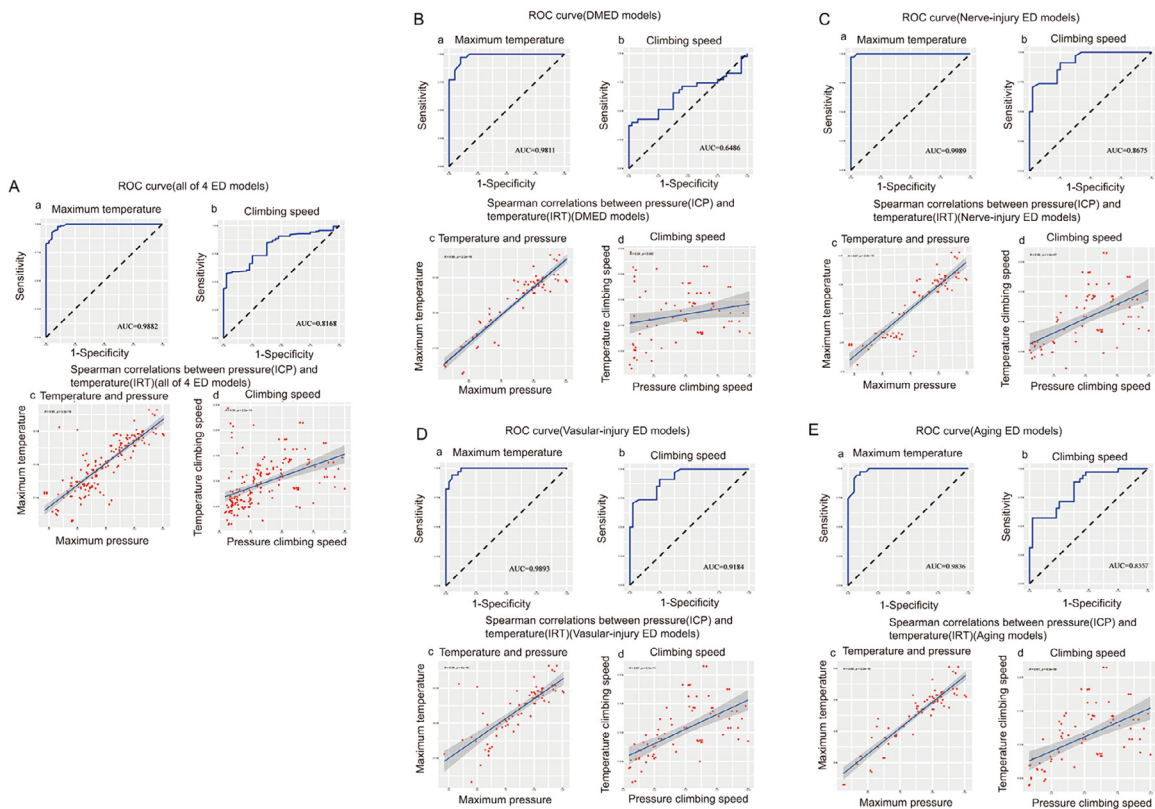


Figure 5. ROC curve and spearman correlation analysis of different models.

A(a,b): The ROC curve of peak temperature and temperature climbing rate in total rats; A(c,d): the scatter diagram of spearman correlation analysis between the values detected by IRT and the values detected by ICP in total rats;
 B(a,b): the ROC curve of peak temperature and temperature climbing rate in DMED models; B(c,d): the scatter diagram of spearman correlation analysis between the values detected by IRT and the values detected by ICP in DMED models;
 C(a,b): the ROC curve of peak temperature and temperature climbing rate in nerve-injury ED models; C(c,d): the scatter diagram of spearman correlation analysis between the values detected by IRT and the values detected by ICP in nerve-injury ED models;
 D(a,b): the ROC curve of peak temperature and temperature climbing rate in vascular-injury ED models; C(c,d): the scatter diagram of spearman correlation analysis between the values detected by IRT and the values detected by ICP in vascular-injury ED models;
 E(a,b): the ROC curve of peak temperature and temperature climbing rate in aging ED models; E(c,d): the scatter diagram of spearman correlation analysis between the values detected by IRT and the values detected by ICP in aging ED models.

improvement might bring progress in eliminate problems in delayed effect between temperature and pressure

To achieve long-term dynamic monitoring, apomorphine¹⁰ was used instead of nerve stimulation to observe the erectile state

of rats in our model system and ideal results similar to ICP were observed. IRT is capable of conducting long-term dynamic monitoring of erectile function in rats, and can be performed several times in a single rat,. Be believed a dynamic monitoring system

Table 1. Spearman correlation analysis

	R value		Diagnostic test of ED with the peak temperature of penis		
	Correlation between peak pressure and peak temperature	Correlation between pressure / temperature climbing speed	Sensitivity	Specificity	AUC P
Total	0.85	0.58	0.98	0.90	0.99 .015
DMED model	0.88	0.24	0.97	1.00	0.99 .023
Aged ED model	0.89	0.61	0.97	0.90	0.98 .041
Nerve- injury ED model	0.87	0.56	0.94	0.95	0.99 .035
Vascular- injury ED model	0.78	0.67	0.97	0.90	0.98 .047

Correlations between the values detected by IRT and the values detected by ICP were evaluated by spearman correlation analysis. Results showed that the peak temperature and temperature climbing rate as detected by IRT showed positive correlation with peak pressure and pressure climbing rate by ICP. Pressure climbing rate =(peak values of ICP-base values of ICP)/ time to peak ICP.

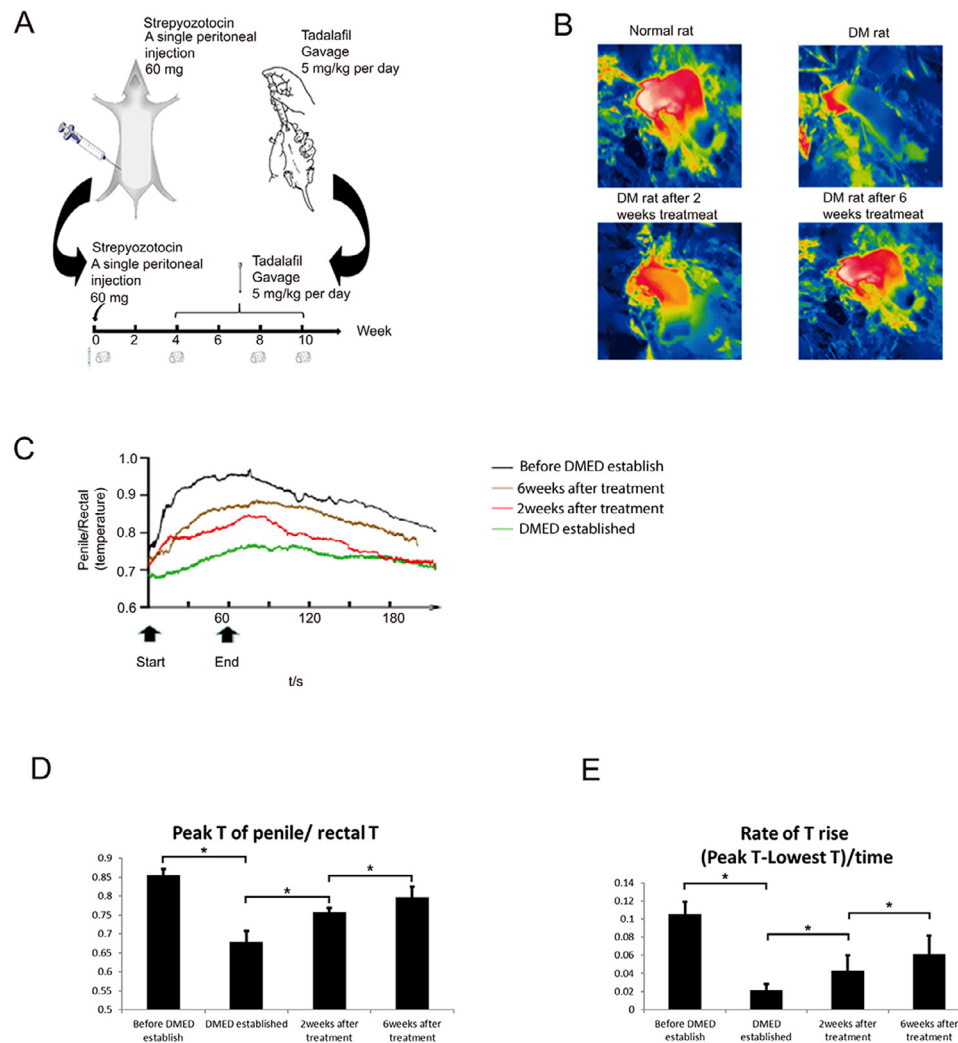


Figure 6. Application of IRT in real-time observation for improving erectile function in a single laboratory animal.

A: Experimental design schema. Long-term monitoring of erectile function was conducted by IRT. Four weeks after STZ injection, DMED in rats was confirmed by thermal imager. During the next 6 weeks, tadalafil was orally given every day at a dose of 5mg/kg. The erectile function of rats was detected by IRT after administration of apomorphine; B: Real-time observation of improved erectile function on a single DMED rat after treatment. C: Penile/Rectal temperature rates exhibited by real-time curves to reflex the improvement of erectile function. D,E: 10 independent experiments were conducted and data was taken into statistical graph. Peak T of penile: Peak temperature of rat's penile. Rate of T rise: Speed of temperature climbing= (Peak temperature –Lowest temperature of penile)/time consumed. * $P < .05$, we compare the multiple groups by the one-way repeated measures anova through SPSS 25.0.

on lab animals bring more helps in drug development and screening

However, IRT has its own disadvantages. Firstly, the technology is vulnerable to environmental factors, such as ambient temperature, humidity and light intensity,¹¹ the experimental conditions were set to reduce this possibility of error. Secondly, hair of the animal reduces the radiation rate, and the roughness of the rat skin also affects. Thirdly, there are individual differences in the body temperature of rats, which requires normalization of rat body temperature with a constant temperature rat platform, and the measured rectal temperatures of rats. These were then compared with that of the penis temperature in attempt to reduce individual bias. In addition, the penile skin temperature

is gradually increased after blood perfusion, which has a certain lag period too.¹¹

In conclusion, a novel noninvasive method for monitoring erectile function in rat ED models was established, which providing a promising replacement or supplement ICP test.

Corresponding Author: Feng Kong, MD, PhD, Department of Urology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 9677 Jing Shi Rd, Jinan, Shandong, P, Jinan, SD 25002, China. Tel: +86053168777945; E-mail: kongfeng@sdu.edu.com

Corresponding Author: Qiang Fu, MD, PhD, Department of Urology, Shandong Provincial Hospital Affiliated to Shandong

First Medical University, 9677 Jing Shi Rd, Jinan, Shandong, P, Jinan, SD 25002, China. Tel: +86053168772911; E-mail: qiangfu68@163.com

Conflict of Interest: The authors report no conflicts of interest.

Funding: National Natural Science Foundation of China (81602227, 81873830, 82071653); Shandong Province Medical and Health Development Plan (202004051394); Jinan Science and Technology Plan (201907037); National Health Commission Medical and Health Development Plan (HDSL2020010640); Jinan new colleges and universities 20 projects (2021GXRC085); Academic Promotion Plan of Shandong First Medical University.

STATEMENT OF AUTHORSHIP

Operation of animal experiment: Shuai Liu, Zhendong Zhao; Formal analysis and investigation: Tongxiang Diao, Ziwen Wang, Dingqi Sun; Writing - original draft preparation: Shuai Liu; Writing - review and editing: Qiang Fu; Experimental design: Shuai Liu; Animal modeling: Shuai Liu, Keqin Zhang, Hui Zhang; Overall management of laboratory equipment: Feng Kong

REFERENCES

- Schoofs E, Fode M, Capogrosso P, et al. Current guideline recommendations and analysis of evidence quality on low-intensity shockwave therapy for erectile dysfunction. *Int J Impot Res* 2019;31:209–217.
- Zhang C, Luo D, Li T, et al. Transplantation of human urine-derived stem cells ameliorates erectile function and cavernosal endothelial function by promoting autophagy of corpus cavernosal endothelial cells in diabetic erectile dysfunction rats. *Stem Cells Int* 2019;2019:2168709.
- Hox M, Mann-Gow T, Lund L, et al. Cavernous nerve stimulation and recording of intracavernous pressure in a rat. *J Vis Exp* 2018(134):56807.
- Chen D, Zhang KQ, Li B, et al. Epigallocatechin-3-gallate ameliorates erectile function in aged rats via regulation of PRMT1/DDAH/ADMA/NOS metabolism pathway. *Asian J Androl* 2017;19:291–297.
- Hu LL, Zhang KQ, Tian T, et al. Probucol improves erectile function via Activation of Nrf2 and coordinates the HO-1/DDAH / PPAR- γ / eNOS pathways in streptozotocin-induced diabetic rats. *Biochem Biophys Res Commun* 2018;507:9–14.
- Dong F, Tao C, Wu J, et al. Detection of cervical lymph node metastasis from oral cavity cancer using a non-radiating, noninvasive digital infrared thermal imaging system. *Sci Rep* 2018;8:7219.
- Law J, Morris DE, Budge H, et al. Infrared thermography. *Handb Exp Pharmacol* 2019;251:259–282.
- Singh D, Singh AK. Role of image thermography in early breast cancer detection- past, present and future. *Comput Methods Programs Biomed* 2020;183:105074.
- Ng YK. A review of thermography as promising non-invasive detection modality for breast tumor. *Int J Therm Ence* 2009;48:849–859.
- Choo SH, Sung HH, Chae MR, et al. Comparisons of apomorphine-induced erection and spontaneous erection in rats by telemetric assessment of intracavernosal pressure. *Andrology* 2015;3:309–314.
- Tavares IM, Vardasca R, Cera N, et al. A review of infrared thermography as applied to human sexual psychophysiology. *Int J Psychophysiol* 2018;133:28–40.