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Research Article

Improving reproductive potential of male albino Wistar *Rattus norvegicus* using Vitamin E and Laserpuncture

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Abstract
Laserpuncture induction and administering Vitamin E as an opulent antioxidant may improve sperm quality. This study aimed to increase rat reproductive potential by inducing laserpuncture and adding vitamin E to their feed. The experimental study adopted a post-test in a completely randomized design in male albino Wistar rats (*Rattus norvegicus*), with four groups (Group A to D) : Group A: the control group (without vitamin E or Laserpuncture), Group B: Administered with vitamin E, Group C: Treated using Laserpuncture induction, and Group D: Treated using Laserpuncture induction and administered with Vitamin E and five replicates. The rat reproductive potential parameters included testosterone levels, Gonado Maturity Stage (GMS), Gonado somatic Index (GSI), and concentration, motility and viability of sperm. Induction of Laserpuncture and administered with Vitamin E influenced testosterone hormone levels, motility viability, and the concentration of male rat's sperm ($P < 0.05$). Another finding showed that the higher GSI was not statistically significant ($P > 0.897$) with mature gonads, and GMS gave the most mature V stage for all treatments. This study concluded that the combination of Laserpuncture induction and administration with Vitamin E had increased the reproductive potential of male albino Wistar rats in terms of testosterone levels and sperm quality.

Keywords: Vitamin E, Laserpuncture, Reproductive potential, Male Wistar rats

INTRODUCTION

Laser (Light Amplification by Stimulated Emission of Radiation) amplifies light derived from electromagnetic wave energy that may induce biostimulation in biological tissues such as gonads (Plavskii *et al*., 2021). Laserpuncture uses nonthermal low-intensity laser irradiation to stimulate acupuncture points as a tool for organ biostimulation (Chon *et al*. 2019). Due to the increased demand for livestock and fishery seeds, the availability of mature gonad broodstock should be improved. Therefore, an attempt to find a solution to increase the availability of superior quality gonad mature broodstock in sufficient quantities is encouraged (Qomar *et al*. 2017; Adikara, 2018; Hariani *et al*. 2020). The use of laserpuncture technology is one approach to overcoming the problem of insufficiency in mature gonadal broodstock. This technology has been applied in some livestock, such as male *Mojosari* ducks (*Anas plathyrhynchos*) (Adikara *et al*. 2017); *Garut* rams (*Ovis aries*) (Herdis, 2010) and some fish species, such as catfish (*Clarias* sp.) (Kusuma and Hariani, 2017), and striped catfish (*Pangasianodon hypophthalmus*) (Mukti *et al*. 2020).

Laserpuncture is one of the applicative technologies used mainly to spur the reproductive potential of livestock and fish has been used to increase gonadal maturity (Mukti *et al*., 2020; Adikara, 2018) and sperm quality rapidly. Gonadal maturity indicators can refer to the Gonado Somatic Index (GSI) and Gonado Maturity Stage (GMS) value (Hariani *et al*. 2020; 2021). The use of laser technology has been successfully applied in one-year-old, weight (1,000–1,200 g) and virgin male sharp-tooth catfish (*Clarias gariepinus* var Mutiara)

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broodstock. The results indicated that induction of Laserpuncture improves male catfish reproductive ability through enhancement of the testosterone level (Hariani *et al*. 2021).

The other approach to accelerate gonad maturation and improve sperm quality can be used by combining Laserpuncture induction with the addition of Vitamin E to the feed. In general, the reproductive potential of animals can be increased by providing quality feed to improve gonad maturation and spermatogenesis (Cheah and Yang, 2011; Pascoal *et al*., 2022), hormonal production such as the steroid hormone (testosterone) for growth and development of the reproductive system (de Oliveira *et al*. 2018), and the quality of sperm that consists of the motility, concentration and viability of sperm to influence the ability of sperm to fertilize the ovum (Esmaeili *et al*. 2015; Suliga and Głuszek 2020; Benatta *et al*. 2020). In addition to feeding, administering Vitamin E can accelerate spermatogenesis (Zubair, 2017). Furthermore, this statement is closely related to intratesticular testosterone levels and fertility in animals such as rats, which can also improve sperm quality (Vahidinia *et al*. 2016).

The albino rat (*Rattus norvegicus*) with the Wistar strain is often used as a model in research that represents mammals' biological system (Domínguez-Oliva *et al*., 2023; Makowska and Weary, 2019). No study has examined the effect of Laserpuncture induction combined with Vitamin E performed at nine acupuncture points at reproductive organs to increase the reproductive potential of male albino Wistar rats, including testosterone levels, sperm quality, GMS, and GSI. Theferfore, the present study aimed to examine the effect of vitamin E, Laserpuncture on stimulating reproductive potential, including concentration, motility, and viability of sperm, GSI, GMS, and testosterone levels in male albino Wistar rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Ethical approval

The Animal Ethics Commission of the Brawijaya University, Malang, Indonesia, approved all animal procedures in this experiment with an ethical approval number of 129-KEP-UB-2022.

Preparations of experimental rats

Twenty male albino Wistar rats, with body weight (BW) of 125-150 g, aged 55-60 days, were collected from the Experimental Animal Development Unit of Gadjah Mada University, Yogyakarta, Indonesia. They were selected based on their health and virginity. The selected rats were acclimatized in cages with dimensions of 30 × 30 × 20 cm/rat/cage made of wood with a wire screen in front, wood shavings on the base, equipped with a place to eat, and the automatic drinker made of plasticine. The experiments were conducted in the Faculty of Sports Science, Universitas Negeri Malang, East Java, Indonesia. They were kept in clean cages under controlled laboratory conditions (temperature 23°-25°C and 12/12 hours of normal light/dark cycles and fed BR2 from Comfeed (Indonesia) standard formula with a crude protein content of 19% as much as 3% of their BW and, as well as drinking water was available *ad libitum* for 7 days.

Laserpuncture application

This study used a semiconductor soft-laser with a power of 20 mW, a 15-second preset laser induction timer with laser induction frequency once every 14 days on day 0 after acclimation, 14, 28, and 42. Laserpuncture treatment was performed at nine reproductive points, namely the two acupuncture points BL 22 (Bladder Meridian) or Sanjiaoshu are located between the processus transversus of the lumbar vertebrae 2 and 3 on the right (dexter) and left (sinister) sides. The two acupuncture points BL 23 (Bladder Meridian) or Shenshu are located between the processus transversus of the lumbar vertebrae 3 and 4 on the right (dexter) and left (sinister) sides. The two acupuncture points GV 4 (Governor Vessel) or Mingmen are located on the dorsal side of the joint between the processus transversus of the lumbar vertebrae 4 and 5 on the right (dexter) and left (sinister) sides and in the testes' upper, middle, and lower ends. Laser induction was carried out on day 0 (the 7^{th} day after acclimation was completed), 14^{th}, 28^{th}, and 42^{nd} days with an induction time of 15 seconds for each point. Total time used 9 points x 15 seconds = 135 seconds/head/per 14 days. For the record, before being induced by the laser, one day previously the rats fasted and their eyes were covered with a small towel to reduce stress for the rats (Hariani *et al*. 2022)

Vitamin E administration

Vitamin E was administered to the rats based on the dose of Vitamin in humans with a BW of 70 kg, namely 600 mg/day of vitamin E (α-tocopherol) tablets 400 mg from Santa-E. The preventive dose was used in humans, and was changed for rats with a BW of 125 g and a conversion factor of 0.018. The dose of vitamin E in rats (125 g) is the human dose x conversion factor = 600 mg/day x 0.018 = 10.8 mg/125 g BW/day, so the dose of vitamin E in rats (per g) was 0.0864 mg/day g BW/day. The maximum oral vitamin E solution was 5 ml/100 g BW; every 86.4 mg solution was dissolved in 1 ml of sesame oil (Arjadi *et al*., 2022). Vitamin E was given in pelleted feed according to the treatment, carried out on day 0 after acclimation was completed until day 41. On day 41 of the experiment, all rats were allowed to fast overnight.

Anesthesia procedure

At the end of the experiment process, the rats were anesthetised using ketamine 100 mg/kg BW (Brand Ivanes) and xylazine 10 mg/BW (Brand Xyla). Both were mixed in a 1:1 ratio. The injection dose was 0.1 mL/rat intramuscularly and waited fainting for about 5-10 minutes until the rats were calm (Schwenk *et al*. 2018; Brown and Tucker, 2020). Subsequently, the rats were dissected on the abdominal part from anal to ventral. Morphological and histological gonadal maturity was observed based on weight and gonadal development through histology preparation. A Gonad sample was prepared according to Junqueira and Carneiro (2005) to measure the GSI. Histological analysis of rat testis was carried out at the Bioscience Laboratory, while testosterone levels were analyzed at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia.

Experimental design

The experimental study used was a post-test experimental with design in a completely randomized design. The sample used 20 male Wistar rats divided into four groups (Group A to Group D). Each group consisted of five rats. The group division was as follows: Group A) pelleted feed as control, Group B) vitamin E in pelleted feed, Group C) pelleted feed combined with laser puncture induction, and Group D) Laserpuncture induction combined with vitamin E in pelleted feed.

Gonadosomatic Index

Rats were anesthetized using Ketamine Brand Ivanes ® dose of 50 mg/kg BW injected intramuscularly and waited to faint for about 5-10 minutes until the rats were calm, then measured their weight using Ohauss® digital animal scales. The rats were then dissected, testicles and epididymis removed and rinsed with physiological saline solution (0.9% NaCl). The body and testis were weighed using Shimadzu's ® digital analytical balance to determine its Gonado Somatic Index based on Ullah *et al*. (2019; 2021). The body weight was used to determine the GSI, and five individuals were taken for further observation.

$$GSI = \frac{Tw}{Bw} \times 100\% \quad \text{Eq. 1}$$

Where

Tw = Testis weight

Bw = Body weight

Testosterone hormone assay and tissue testis processing

Testosterone levels.

Blood samples were taken directly from the heart with a syringe and were collected and transferred into a non-anticoagulant tube at a volume of 5 mL. The blood was kept in Eppendorf tubes and stored at room temperature with a tilt of 45° for half to an hour for serum separation. Then, blood was centrifuged Costar® at 1,500–3,000 rpm for 10 minutes at 4°C. The serum was collected using a micropipette and transferred to a labeled Eppendorf tube. Serum samples were stored at −20°C. The serum was assayed using an Enzyme-linked immunosorbent assay (ELISA) kit to determine testosterone (T) (Elisa Kit cat. No. MDEL0318Ge) using the suggested assay protocol in the kit (Hayati *et al*. 2018). The levels are obtained according to the manual from the kit and read using an ELISA reader Agilent BioTek Synergy Neo2 Hybrid Multimode Reader ®- at a wavelength of 450 nm (Lee *et al*. 2022).

Testis tissue processing and gonad maturity stage observation

The testis collection was conducted on the same day as the blood sample collection. The testicular organs were washed in physiological NaCl, then fixed using a 10% Neutral Buffer Formalin solution with pH 2.7 to 4.7 for 48 hours (Hopwood, 2002). After 48 hours, the organs were processed using 70 % alcohol and the dehydration process using graded alcohol (70%, 80%, 90%, Absolute) was repeated twice, each for 30 minutes. The clearing process is carried out using xylol: paraffin (ratio 1 : 3, 1 : 1, 3 : 1) for 30 minutes each, followed by the infiltration process using liquid paraffin and the embedding process using paraffin block. Cutting organs using a Rotary Microtome Cut 6062 - SLEE MEDICAL GMBH – Germany device with a 3-5 µm thickness. The testicular pieces were put on the glass object and smeared with albumin-glycerin (37 - 38ºC), then dried using a Hot Plate at 35ºC for 3 minutes and stained using Hematoxyline-Eosin staining was conducted thereafter and then sealed with Entellan® (Merck Millipore, Germany) (Bancroft and Gamble, 2007).

Each histological preparation of the testis was documented using a microscope (Olympus CX31, Japan) connected to a digital camera. The number of cells were counted based on image processing (Miconos, Yogyakarta, Indonesia). Assessment of the number of sertoli cells, leydig cells, and spermatogenic cells was conducted according to Tu *et al*. (2011). The cells were observed using an optical microscope with 1000 × magnification. Based on the indicators of gonadal maturity according to the Johnsen method, it could be categorized as 10 (Johnsen, 1970). The interpretation used Johnsen's (1970) method of five fields of view in each replication with 400 magnifications. The scoring categories were: 1) no germ cells and Sertoli cells; 2) no germ cells; 3) there are only spermatogonia; 4) there are only a few spermatocytes; 5) no spermatozoa and spermatid cells, but many spermatocytes; 6) few spermatid cells; 7) no spermatozoa, but many sperma-

tid cells; 8) a few spermatozoa cells; 9) many spermatozoa but unorganized spermatogenesis; 10) complete spermatogenesis and tubular seminiferous.

Sperm quality

Collection of Sperm from the rat epididymis. The right and left cauda epididymis were incised. Then, both were placed on a petri dish containing 1 mL of 0.9% physiological NaCl for stripping using tweezers to remove sperm so it is suspended in 0.9% physiological NaCl solution. The solution must be mixed until homogeneous.

Sperm concentration and observation

The 10µL of sperm suspension was isolated and put into the Assistant Improved Neubauer hemocytometer made in German boxes, covered with a cover slip, and then counted (Khak *et al*. 2009). Sperm concentration was calculated according to Harper *et al*. (2020) under a Nikon Eclipse E100 light microscope with 400 × magnification.

Sperm motility and observation

The sperm suspension was taken and dripped and dripped onto the object glass, then covered with a cover glass. Motility observations were conducted under a Nikon Eclipse E100 ® light binocular microscope with 400 × magnification. Observations were made at least in five visual fields. Each field of view was recorded for 20 seconds. Afterwards, a sperm motility classifier was carried out, including progressive motility (sperm moving fast and straight) and non-progressive (sperm moving in place, circular or backward). The evaluation of sperm motility was undertaken by comparing the progressive sperm that moved forward with those that move backward or stayed in place. Calculation of the percentage (%) of motility was carried out by two estimators (Sousa *et al*. 2013).

$$MP = \frac{MS}{TS} \times 100\% \quad \text{Eq. 2}$$

where
MP = Motility Percentage (%)
MS = Number of spermatozoa moving forward
TS = The total number of spermatozoa

Sperm viability and observation

Determination of spermatozoa viability was carried out using the eosin-negrosin staining technique (4:1) mixed with sperm suspension (4:1) and smeared on an object glass with an inclination of 45^0. Then it was dried. The viability percentage was calculated under a Nikon Eclipse E100 light microscope binocular with 400x magnification to observe live and dead spermatozoa. Those that did not absorb color were live sperms, while dead sperms absorbed dye. Calculating sperm viability according to Sousa *et al*. (2013) as follows:

$$V = \frac{VS}{TS} \times 100\% \quad \text{Eq. 3}$$

where
V = Viability Percentage (%)
VS = Number of viable spermatozoa
TS = The total number of spermatozoa

Data analysis

Development stages of gonad maturity testicular histology were analyzed by using descriptive statistics. Sperm quality, testosterone hormone levels, and GSI were analyzed using analysis of variance (ANOVA) with SPSS 16 software. If the results were significant, the analysis continued with Duncan's multiple range test with a 95% confidence level.

RESULTS

Testosterone hormone levels

The study showed that all treatments (Group A to D) had improved testosterone production. Treatments of Laser (Group C) and Laser with vitamin E administration (Group D) had affected testosterone production significantly ($P < 0.05$), while that of vitamin E administration alone did not affect testosterone production significantly ($P > 0.05$). Treatment of vitamin E administration combined with Laserpuncture induction produced the highest testosterone level (19.54 ng/mL), while that of vitamin E administration alone produced the lowest testosterone level (7.89 ng/mL) (Fig. 1).

Development of Gonadal Maturity Stage (GMS) and Gonado Somatic Index (GSI) of male Wistar albino rat

The body weight (BW) of male Wistar rats early in life before sexual maturity (55–60 days of age) was relatively the same. As age increases (14-16 weeks), the adult male rats became sexually matured and ready to mate. The indicators used for gonad maturation were the Gonado Maturity Stage (GSM) and the Gonado Somatic Index (GSI). Testis of male Wistar rats after an experiment carried out for 42 days in spermatogenesis contained stages of sperm development.

The results of histological preparations demonstrated the process of spermatogenesis, which is indicated by a stage of sperm development. The results of this study showed that in the control (Group A), given vitamin E in pelleted feed (Group B), laserpuncture induction (Group C), or given vitamin E combined with laser puncture induction (Group D) there were all types of spermatogenic cells in the Tubuli seminiferous of the testes, namely spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and sperm. In addition, Sertoli cells and Leydig cells are also present.

Table 1. Developmental stages of spermatozoa, Sertoli and Leydig cells (mean ± SD) after treatments

Treatments	Number of cells						
	Spermatogonia	Primary spermatocyte	Secondary spermatocyte	Spermatid	Sperm	Sertoli cells	Leydig cells
Group A:C	60 ± 2	124 ± 23	4 ± 3	148 ± 19	254 ± 60	7 ± 1	19 ± 6
Group B: Vit E	56 ± 5	154 ± 17	5 ± 2	155 ± 26	263 ± 23	6 ± 1	25 ± 5
Group C:L	49 ± 7	173 ± 19	8 ± 1	162 ± 13	271 ± 31	3 ± 1	30 ± 4
Group D:L & Vit E	43 ± 7	181 ± 11	5 ± 1	183 ± 17	370 ± 22	2 ± 0	35 ± 4

Note: C = Rats fed pellets (control); Vit E = administration of vitamin E; L = laser induction

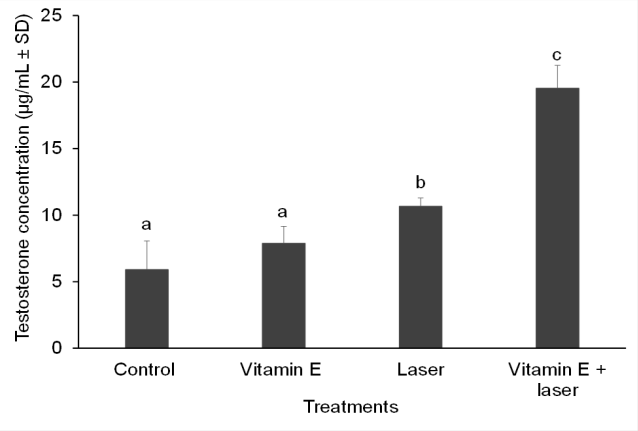

Fig. 1. *Mean ± SD testosterone levels (ng/mL) among the treatments in male Wistar rats. Note: The mean and standard deviation values in the column followed by different superscript letters indicate significantly different; Control (rats feed with pellet - Group A), Vitamin E (rats feed with pellet and vitamin E supplement - Group B), Laser (rats feed with pellet and induced by Laserpuncture - Group C), Vitamin E and Laser (rats feed with pellet, vitamin E supplement and induced by Laserpuncture – Group D)*

The presence of five spermatogenic cells and a more significant number of sperm cells can be categorized as mature gonads. The result showed that a combination treatment of laser induction and vitamin E produced the highest number of Primary spermatocyte, Spermatid and Spermatozoa cells (Table 1). The photo observation of gonadal histology preparations (testes) presented in Fig. 2. Confirmed the data of sperm development stages.

This study showed that Laserpuncture treatment affected the gonadal development of male rats, as seen in Fig. 2. The results of this study indicated that the control group, vitamin E in feed, laser induction, or given vitamin E in feed combined with laser induction had all types of spermatogenic cells in the seminiferous tubules of the testes, namely spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperm. Sertoli cells and Leydig cells were also present. With five spermatogenic cells and a higher number of sperm cells, the gonads were categorized as mature. Sertoli was also more associated with nutrition. The difference was the condition of the number of spermatids and sperm was greater. Those given vitamin E treatment combined with laser induction had a more significant number, accompanied by many Leydig cells. It was followed by laser induction treatment and a vitamin E in feed as the new control group. Based on the indicators of gonadal maturity according to the Johnsen method, it could be categorized in a value of 10 (Table 1) and confirmed by the results of photos of histological preparations of the gonads (testis) presented in Fig. 2.

The study showed that all treatments had improved GSI value, but no significant effect was not observed in all treatments ($P > 0.05$). Treatment of vitamin E administration combined with Laserpuncture induction produced the GSI value (1.22), while that of vitamin E administration alone produced the lowest GSI value (1.13) (Fig. 3).

Sperm quality

The study showed that all treatments had improved sperm concentration. Treatments of vitamin E administration combined with Laserpuncture induction had affected the sperm concentration significantly ($P < 0.05$), while the other treatments did not affect the sperm concentration significantly ($P > 0.05$). Treatment of vitamin E administration combined with Laserpuncture induction produced the highest sperm concentration (22.48×10^6 /mL), while that of vitamin E administration alone produced the lowest sperm concentration (17.08×10^6 /mL) (Fig. 4A).

All treatments had improved sperm motility. Treatments of both Laserpuncture and combination of vitamin E administration with Laserpuncture induction had affected the sperm motility significantly ($P < 0.05$), while vitamin E administration alone did not affect sperm motility significantly ($P > 0.05$). Treatment of vitamin E administration combined with Laserpuncture induction produced the highest sperm motility (77.4%), while that of vitamin E administration alone produced the lowest sperm motility (39.7%) (Fig. 4B).

All treatments had improved sperm viability significantly ($P < 0.05$). Treatment of vitamin E administration combined with Laserpuncture induction produced the highest sperm viability (83 %), while that of vitamin E ad-

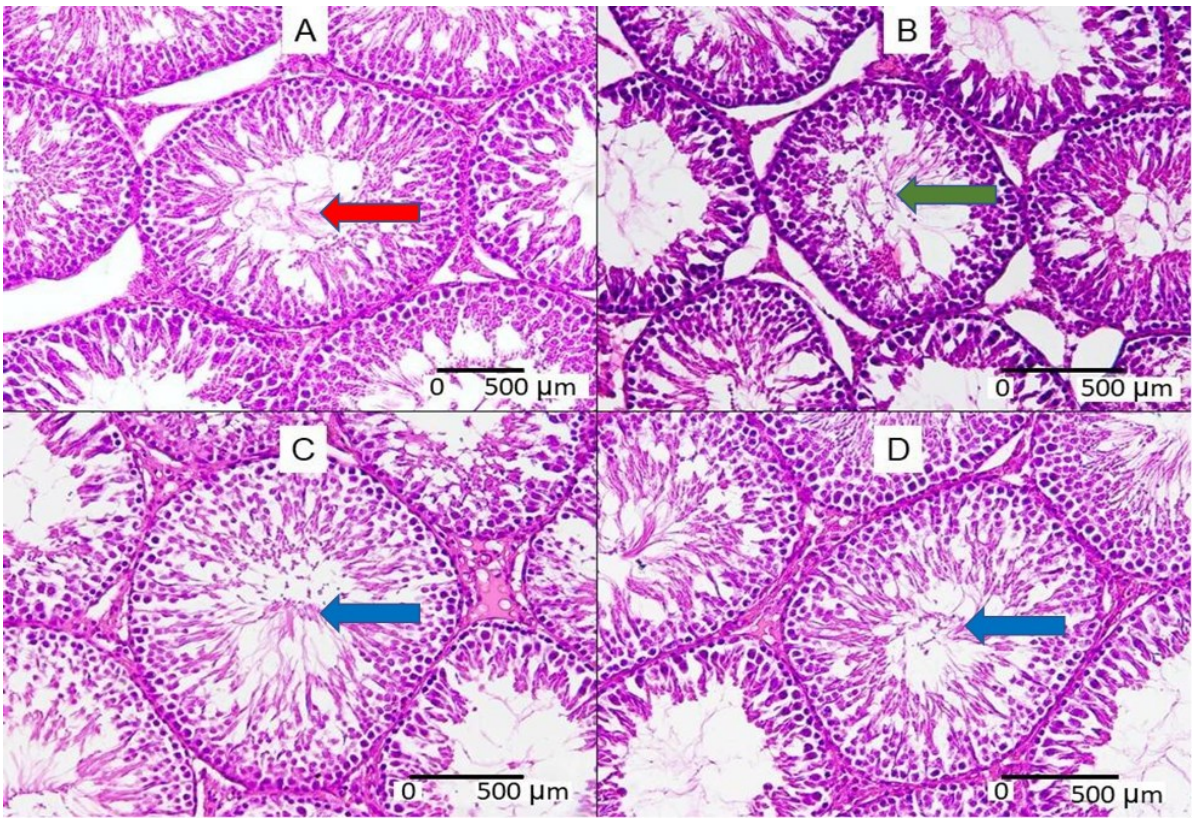

Fig. 2. *Showing testicular cells of male Wistar rats after being treated with vitamin E and induction of laserpuncture. Note: Control (rats feed with pellet - Group A) showing complete spermatogenesis with spermatozoa in the center of lumen (red arrow); Vitamin E (rats feed with pellet and vitamin E supplement - Group B) showing completed spermatogenesis with more spermatozoa in the center of lumen than Group A (green arrow); Laser (rats feed with pellet and induced by Laserpuncture - Group C) showing complete spermatogenesis with rapid spermatozoa (blue arrow); Vitamin E and Laser (rats feed with pellet, vitamin E supplement and induced by Laserpuncture – Group D) showing complete spermatogenesis with rapid spermatozoa (blue arrow).*

ministration alone produced the lowest sperm motility (72 %) (Fig. 4C).

DISCUSSION

This study showed three significant findings. First, the combination of Laserpuncture and vitamin E (Group D) induction at the reproductive point has increased all observed reproductive parameters of Wistar rats, especially testosterone levels and sperm quality, except GSI. The values of all parameters resulting from this treatment were consistently higher than the control group without vitamin E or Laserpuncture (Group A), and those treated with vitamin E (Group B) or Laserpuncture (Group C) alone. Second, the application of laserpuncture (Group C) has affected the reproductive system of rats, resulting in higher observed parameter values than the administration of vitamin E (Group B). Third, administering vitamin E (Group B) increased sperm viability, but no significant effects were observed on other parameters. The combination of Laserpuncture and vitamin E (Group D) induction has significantly increased several reproductive parameters of male albino Wistar rats compared to other treatments and controls. This implies that the combination of Laserpuncture and vitamin E (Group D) induction is synergistic. This combination was able to increase testosterone levels in male Wistar albino rats to produce the highest levels (19.54 ng/mL) compared to other treatments and controls. It can be seen that this treatment (Group D) can increase testosterone levels by around 400%. Other studies have also shown that administering Laserpuncture with a duration of 5-20 seconds/rat/day carried out for a week showed increased testosterone levels in rats (Purnama et al. 2018).

The method developed in this study is different from that of previous studies. The difference with previous studies is the location of Laserpuncture induction, the duration of Laserpuncture induction, and the length of induction days. This study uses a very short time of only 15 seconds, while previous studies used a very long induction time; for example, in the study of Kizilay *et al*. (2023), induction was carried out for 30 minutes. In this study, laserpuncture induction was carried out at a less frequent frequency (every two weeks) compared to previous studies, which had a frequency of observa-

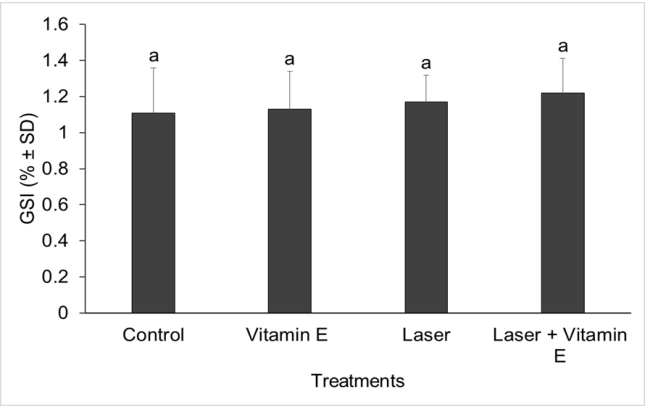

Fig. 3. *Mean ± SD GSI in male Wistar rats among the treatments. Note: Control (rats feed with pellet - Group A), Vitamin E (rats feed with pellet and vitamin E supplement - Group B), Laser (rats feed with pellet and induced by Laserpuncture - Group C), Vitamin E and Laser (rats feed with pellet, vitamin E supplement and induced by Laserpuncture – Group D). Mean and standard deviation values in the column followed by different superscript letters indicate significantly different*

tion once a week (Purnama *et al*., 2018). Therefore, Laserpuncture induction with a lower duration and frequency is quite effective and efficient in producing and increasing testosterone hormone.

Laser is an electromagnetic wave energy that can cause biostimulation in biological organs such as fish (Plavskii *et al*., 2021). Low-power lasers (soft lasers) can be used to influence cell metabolism (Mukti *et al*., 2023). Laser induction can affect brain tissue's physiological reactions that activate the Glutamic Acid Decarboxylase-67 (GAD-67), stimulating GABAergic neurons to synthesize Gamma Amino Butyric Acid (GABA). GABA will stimulate hypothalamic neurons and pituitary neurons (Kusuma and Hariani, 2017) to release Gonadotropin Releasing Hormone (GnRH) so that pituitary neurons release FSH and LH which play a role in spermatogenesis to produce testosterone (Lee et al., 2022). FSH stimulates the development and proliferation of Sertoli cells to produce Androgen Binding Protein (ABP), stimulating spermatogonia to start the spermatogenesis process. LH functions to stimulate interstitial cells or Leydig cells to release testosterone. Testosterone and ABP together control sperm formation.

Furthermore, in the process of spermatogenesis and facilitating the initiation of spermatogenic development, spermatogenesis and facilitating the initiation of spermatogenic development, the gonads become more mature and faster due to Laserpuncture induction (Kizilay *et al*., 2023). This study shows that applying Laserpuncture (Group C) produces higher observed parameter values than administering vitamin E (Group B). Laserpuncture induction works through neurons so that the process is faster than administering vitamin E through the bloodstream (hormonally slower). A higher

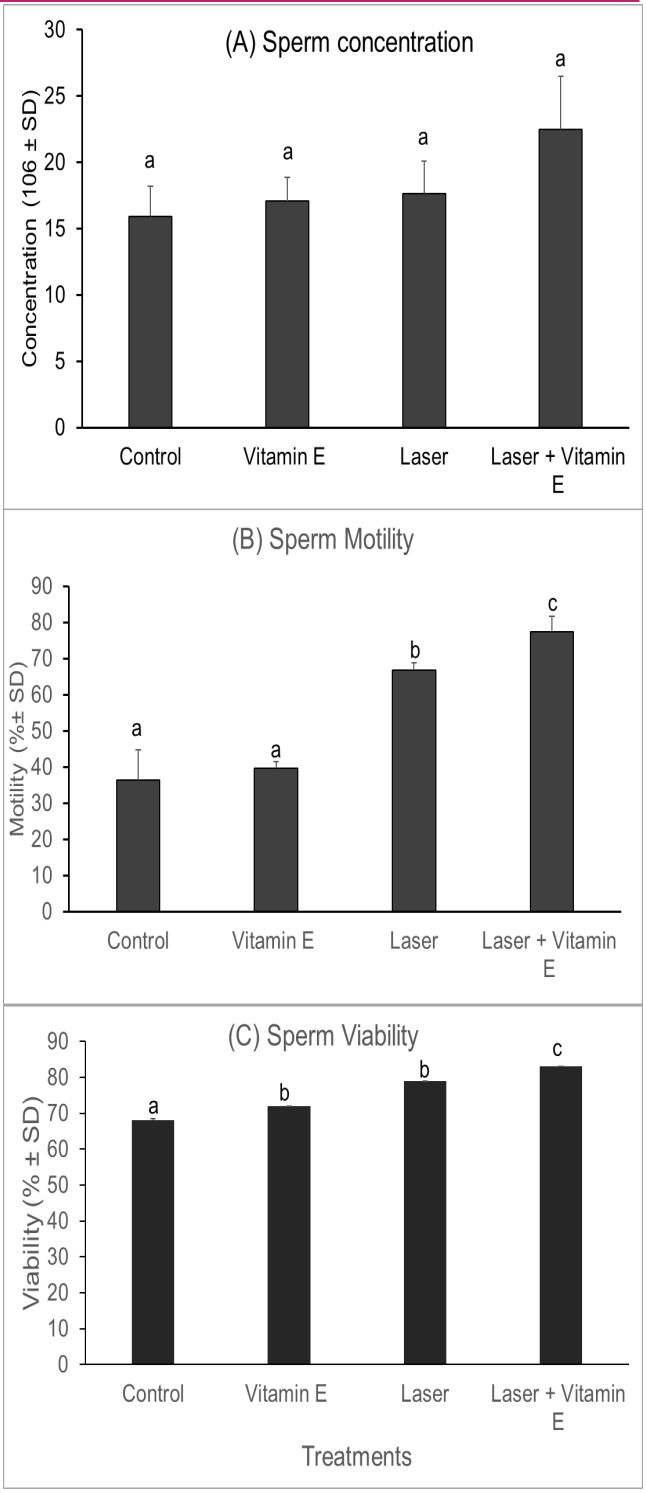

Fig. 4. *Male Wistar rat sperm quality (A) Mean ± SD value of sperm concentration, (B) Mean ± SD value of sperm motility and (C) Mean ± SD value of sperm viability among the treatments. Note: Control (rats feed with pellet - Group A), Vitamin E (rats feed with pellet and vitamin E supplement - Group B), Laser (rats feed with pellet and induced by Laserpuncture - Group C), Vitamin E and Laser (rats feed with pellet, vitamin E supplement and induced by Laserpuncture – Group D); Mean and standard deviation values in the column followed by different superscript letters indicate significantly different*

combined effect indicates the synergistic nature of the two factors compared to the effect of a single factor. Male rat from the combination treatment group (Group D) have a GSI value. The results of mouse testes' histological preparations show all stages from germ cells to spermatozoa. The results showed that the testes of male albino Wistar rats in all treatments contained all stages of sperm development. However, in the control group, the number of spermatogonia cells was greater, the number of Sertoli cells was relatively greater, and the number of spermatozoa cells and Leydig cells was relatively less than in the treatment group. In the combination treatment (Group D), the number of spermatogonia, primary spermatocyte, secondary spermatocyt, spermatid, spermatozoa, and Leydig cells was relatively greater than in the other treatments (Table 1).

The results of the study for GSI were not much different from GSM. The results of the ANOVA test showed that administering Vitamin E combined with Laserpuncture induction in male albino Wistar rats at nine reproductive points could increase GSI, although statistically not significant ($P>0.05$) compared to other treatments. This shows that administering vitamin E or Laserpuncture either singly or in combination does not significantly affect the maturity of rat gonads. This study found that the administration of vitamin E (Group B), laser puncture (Group C), or a combination of both (Group D) also increased the concentration, motility and viability of sperm produced by the testes. These results are in line with previous studies that reported that the administration of vitamin E, laser puncture induction, or a combination of both also increased the number or concentration of sperm produced by the testes (Zubair 2017; Cui et al. 2019), .Concentration or number of sperm produced from the combination treatment (Group D) increased significantly (Zubair 2017; Cui *et al*., 2019). Gentle laserpuncture induction has significantly improved sperm quality, such as sperm concentration, motility, and viability (Cui *et al*., 2019). In this study, the highest sperm concentration, motility, and viability were obtained in the combination treatment (Group D) because this dose can provide a fairly large stimulation effect. This dose can provide a large stimulation effect to stimulate hypothalamic neurons to release GnRH (Gonadotrophin gonadotrophin-releasing hormone). GnRH stimulates anterior pituitary neurons to release FSH Follicle Stimulating Hormone) and LH (Luteinizing Hormone) in mice and activates reproduction (Duittoz *et al*., 2021) by controlling testicular function (Holyoak and Ma, 2022). FSH stimulates Sertoli cells in the seminal tubules to increase Androgen Binding Protein (ABP). ABP is a glycoprotein that binds testosterone and is secreted into the lumen of the seminiferous tubules in high concentrations. Due to the presence of ABP, this can stimulate the growth and development of these cells by releasing various spermatogenic elements. Testosterone that diffuses into the seminiferous tubules of Leydig cells in the interstitial space has a strong trophic effect when FSH and testosterone control spermatogenesis. FSH and testosterone control spermatogenesis. Testosterone and ABP hormones control spermatogenesis and initiate spermatogenic development into motile spermatozoa (Moskvin and Apolikhin, 2018). FSH also plays a role in stimulating Sertoli cell division, maturation, secretory capacity, and cytoskeletal regulation. In the case of germ cells, these actions include stimulation of spermatogonia division and meiosis. Testosterone plays an important role in spermiogenesis.

Nutrients in feed are used for metabolic activities for the survival, growth, and development of the gonads (testes), as well as raw materials for the formation of hormones such as reproductive hormones. This is in line with the work expressed by Benatta et al. (2020) state that the reproductive potential of mice can be increased by providing nutrients in the feed that can affect spermatogenesis so that the quality of sperm produced, both in terms of quantity and concentration, is large and accompanied by high fertility. In line with this, Inzaghi et al. (2022) explained that nutrition in feed plays an important role in regulating the growth and development of animal life, which is mediated by hormonal signals such as steroid hormones. Sex steroid hormones such as testosterone are important components in the main endocrine regulation for the growth and development of the reproductive system. The growth and development of the reproductive system can be influenced by nutritional status.

Conclusion

This study concluded that the treatment of combination of Laserpuncture induction and vitamin E had increased the best reproductive potential of male albino Wistar rats, such as testosterone levels and sperm quality, compared to other treatments and control. The values of all parameters resulting from this treatment were always higher than those treated with vitamin E or Laserpuncture. The application of Laserpuncture has affected the reproductive system of rats to produce higher observed parameter values than the administration of vitamin E. The administration of vitamin E increased sperm viability, but no significant effects were observed on other parameters like GSI. with mature gonads, and GMS gave the most mature V stage for all treatments. Laserpuncture induction with a lower duration and frequency is quite effective and efficient in producing and increasing testosterone hormone. This study recommends that data collection be carried out every 14 days for 42 days for all treatments to observe a more detailed development profile of

reproductive organs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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