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Administration of ethanol extract of young papaya seeds (*Carica papaya L.*) in young adult mice (*Mus musculus*) causes lower spermatozoa quality compared to control

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Abstract: This study aims to determine whether the ethanol extract of papaya seeds (Carica papaya L.) can affect the quality of spermatozoa in young adult mice (Mus musculus). An experimental study was conducted using a post-test only control group design. The sample consisted of 36 male mice (Mus musculus) of the Swiss Webster strain, aged 2-3 months, with a body weight of 20-30 grams. The mice were divided into two groups: the control group (P0), which included 18 male mice given 3% Tween as much as 0.5 ml, and the treatment group (P1), which included 18 male mice given ethanol extract of young papaya seeds (Carica papaya L.) at a dose of 0.21 mg/gram body weight, administered orally at 0.5 ml for 36 days. The data obtained were analyzed using the independent sample t-test. This study is a laboratory experiment designed to evaluate and examine the significance of young papaya seed extract in affecting spermatozoa quality.

Keywords: *Male contraceptives, Spermatozoa quality, Young papaya seed extract.*

1. Introduction

Indonesia is one of the countries that has the largest population in the world due to its high growth rate. This increasing population growth will become a population problem that must be addressed by the government through the National Population and Family Planning Agency (BKKBN) program, namely Family Planning (KB) [1].

The family planning program itself is said to be successfully associated with the use of contraceptives, where women's involvement in using contraceptives is higher than that of men. The contraceptive method for men still has many shortcomings that exist, including now are vasectomy, condoms, and intercourse. Vasectomy is a fairly effective contraceptive, but it has its drawbacks of being invasive and expensive [2]. For this reason, it is necessary to develop contraceptives that quickly reach azoospermia and are *reversible*, do not interfere with libido, and have minimal side effects [3].

The World Health Organization (WHO) is developing male contraceptives from herbs because they have the advantages of low cost, being safe, easy to obtain, low toxicity, and minimal side effects [4]. Indonesia itself has many types of herbal plants that are widely used, so they provide benefits for health development. One of them is the Papaya Plant, which grows a lot in Indonesia, which is predicted to have an antifertility effect [5]. The Papaya plant contains polysaccharides, vitamins, minerals, enzymes, proteins, alkaloids, glycosides, fats and oils, lectins, saponins, flavonoids, tannins, and sterols. Meanwhile, Papaya seeds themselves have secondary metabolites, namely alkaloids, flavonoids, tannins, and saponins, which are predicted to have potential as antifertility.

The flavonoid compounds contained in Papaya seeds are predicted to have antifertility effects by

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inhibiting the function of the hypothalamic-pituitary-gonadal axis, so that spermatogenesis can be disrupted because they can be phytoestrogens [6].

Hexane Fraction and Methanol Fraction Young Papaya Seed Extract May Inhibit Spermatids in Male Mice [9]. Research by Dewanti, et al. [1]. Using ethanol extract of Papaya seeds (*Carica papaya* L.) given to male rats showed a decrease in spermatozoa count and spermatozoa viability, and can reduce the weight of testicles and seminal vesicles with significant results. Based on the above review regarding the lack of male participation in using contraception, the limited male contraceptives available, and the chemical content of papaya seeds has the potential to be antifertile, a study was conducted on giving ethanol extract of papaya seeds (*Carica papaya* L.) to mice (*Mus musculus*). Young adults have lower sperm quality than controls. In this study, a more complete analysis of spermatozoa quality parameters, namely viability, mortality, and morphology of mouse spermatozoa (*Mus musculus*). The spermatozoa quality parameters in this study used the latest criteria from Wiryawan, et al. [4]. The results of the research on young papaya seed extract on spermatozoa quality in experimental animals will be published in the journal Bali Medical Jurnal, which has been indexed by Scopus as the main output, and IP will also be made as an additional output. This research is by the Research Master Plan (RIP) of Udayana University, especially in the field of health and medicine [7].

This study aims to determine the effect of administering ethanol extract of young papaya seeds *(Carica papaya L.)* on spermatozoa quality in young adult mice (Mus musculus). Specifically, this study will test whether the administration of the extract can lead to a decrease in spermatozoa concentration compared to the control group, as well as decrease the motility, viability, and number of spermatozoa with normal morphology.

2. Method

In this study, a purely experimental research design was used, using *a post-test only control group design* [8]. The scheme in this study is described as follows.

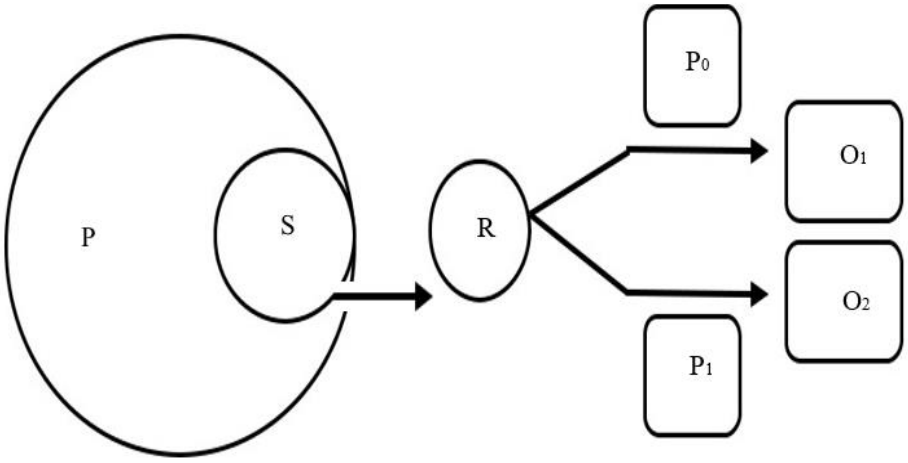

Figure 1.
Research Design.

Information:
P: Population
S: Sample
R: Random
P0: Control group, a group of young adult mice (*Mus musculus*) with administration *Tween* 3% as much as 0.5 ml/day orally for 36 days.

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P1: Young adult mouse (*Mus musculus*) treatment group with administration ethanol extract Papaya Leaves (*Carica Papaya L.*) dissolved with 3% *tween* as much as 0.5 ml at a dose of 0.21 mg/gram BB orally for 36 days
O1: Results of examination of sperm concentration, motility, morphology, and viability in the Group control after treatment for 36 days
O2: Results of sperm concentration, motility, morphology, and viability in the group P1 after treatment for 36 days.

The implementation of making young Papaya seed extract (*Carica papaya* L.) in this study took place at the Food Technology Laboratory, Udayana University. The maintenance and treatment of experimental animals, as well as the observation of the quality (concentration, motility, viability, and morphology) of mouse spermatozoa (*Mus musculus*) in this study took place at the UPT Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Division of Drug Development and Experimental Animals. The implementation of this research was carried out for 6 months starting from March 2024 to February 2025 after obtaining *ethical clearance* for the research.

The target population for this experimental researcher was all male mice (*Mus musculus*) with *the Swiss Webster strain.* The affordable population in this study was male *Mus musculus* with the *Swiss Webster* strain weighing between 20-30 grams and mice aged 2-3 months. In this study, samples were taken from mice (*Mus musculus*) that met the criteria. The inclusion criteria in this study included Swiss Webster strain mice (Mus musculus) that were 2–3 months old, weighed 20–30 grams, and were in good health. Meanwhile, the exclusion criteria include mice that do not want to eat or drink, or have congenital abnormalities or abnormalities.

The number of samples from each treatment group will be calculated using Federer's formula, where in this study there are 2 (two) treatment groups, then:

$(n-1)(t-1) \geq 15$
$(n-1)(2-1) \geq 15$
$n \geq 16$

information:
t: Number of groups = 2 groups, n: Number of samples

Based on the calculation of the sample, the minimum number of samples used in this study is 16 mice per group, so that for the purpose of treatment for 2 groups, 32 are needed with the addition of an anticipation of 10% *drop out,* which is 4 heads. The total sample in this study was 36 mice.

In the Swiss Webster Strain *mouse population,* sample selection was carried out based on inclusion criteria and exclusion criteria. Samples that have met the requirements were then taken at random, as many as 36 *Swiss Webster strain* mice. From the samples that had been selected, they were then divided into 2 groups at random, namely the control group (O1), the treatment group (O2), with each group numbering a sample, namely 18 mice.

Mice (*Mus musculus*) are prepared as many as 36 heads, of which mice are 2-3 months old and weigh 20-30 grams. All mice were adapted for 7 days before being given treatment, On the 8th day, all mice (*Mus musculus*) were divided into two groups, namely the control group (P0) and the treatment control group (P1). All groups of mice (*Mus musculus*) are given treatment according to their group, namely:

1. Control group (P0): In the control group, 3% *tween* was given 0.5 ml orally using a sonde at 11.00-12.00 WITA during the 36-day treatment process. After 36 days of treatment, all mice in the control group were taken from the *cauda epididymis* to be examined for spermatozoa concentration, morphology, motility, and viability. Previously, anesthesia was carried out using Ket-A and Xyla (1:1) as much as 0.2 ml intramuscularly (IM) in the thigh area of the mice, then after the mice were unconscious, the were positioned as comfortably as possible. Next, careful surgery is carried out to remove the *cauda epididymis.* The sample obtained from *the cauda epididymis* was placed on a *glass object* and then observations related to concentration, morphology, motility and viability were carried out under a light microscope with a magnification of 400 times. This examination is carried out by

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qualified experts in this field.

2. Treatment group (P1). In the treatment group, ethanol extract of young Papaya seeds (*Carica Papaya L.*) as much as 0.5 ml with a dose of 0.21 mg/g BB orally using a sonde at 11.00-12.00 WITA during the 36-day treatment process. After 36 days of treatment, all mice in the treatment group were taken from the *epididymis* to be examined for spermatozoa concentration, morphology, motility, and viability. Previously, anesthesia was carried out using Ket-A and Xyla (1:1) as much as 0.2 ml intramuscularly (IM) in the rat thigh area, then the mice were unconsciously positioned as comfortably as possible. Next, careful surgery is carried out to remove the *cauda epididymis*. Samples obtained from the *cauda epididymis* were placed on glass objects and then observations related to concentration, morphology, motility, and viability were carried out under a light microscope with a magnification of 400 times. This examination is carried out by qualified experts in this field.

In this study, the *software application statistical product and service solutions* (SPSS) with version 21.0 [1] was used.

3. Result

This study aims to determine the effect of ethanol extract of Papaya seeds (Carica papaya L.) can reduce the quality of spermatozoa in menicite (Mus musculus) in young adults. In this study, an experimental study was used with *a post-test only control group design* research design. The sample used was 36 male mice (Mus musculus) of the Swiss Webster strain aged 2-3 months with a body weight of 20-30 grams. The sample was divided into 2 groups, namely the control group (P0) consisting of 18 male mice given Tween 3% as much as 0.5 ml and the treatment group (P1) consisting of 18 male mice given ethanol extract of Young Papaya Seeds (Carica papaya L.) 0.21 mg/gram BB as much as 0.5 ml for 36 days orally.

The research was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University and in accordance with the sample criteria that have been determined in the study. This research has received *ethical clearance* from the Research Ethics Commission (KEP) of the Faculty of Medicine, Udayana University/Sanglah Central General Hospital, Denpasar, Bali.

Data on the concentration, motility, viability and normal morphological number of spermatozoa were tested for normality using the Shapiro-Wilk test. The results showed that the normal concentration, motility, viability and morphology of spermatozoa were normally distributed ($p>0.05$) (Appendix 3). Data on the concentration, motility, viability and normal morphological number of spermatozoa were tested for homogeneity using the Levene's test and showed homogeneous data ($p>0.05$) (Appendix 3).

The comparability analysis aimed to determine the comparison of Spermatozoa concentration between the control group (P0) and the treatment group (P1). The results of the significance analysis with the t-independent test are presented in Table 1.

Table 1.
Difference in Average Spermatozoa Concentration between the Control Group (P0) and the Treatment Group (P1).

Group	*n*	Rerata ±SB (million/ml)	*p*
Control (P0)	18	17.100±2.1828	0.000
Treatment (P1)	18	11.722±1.9157	

Description: *n* = amount of data SB = Baku junction *p* = significance

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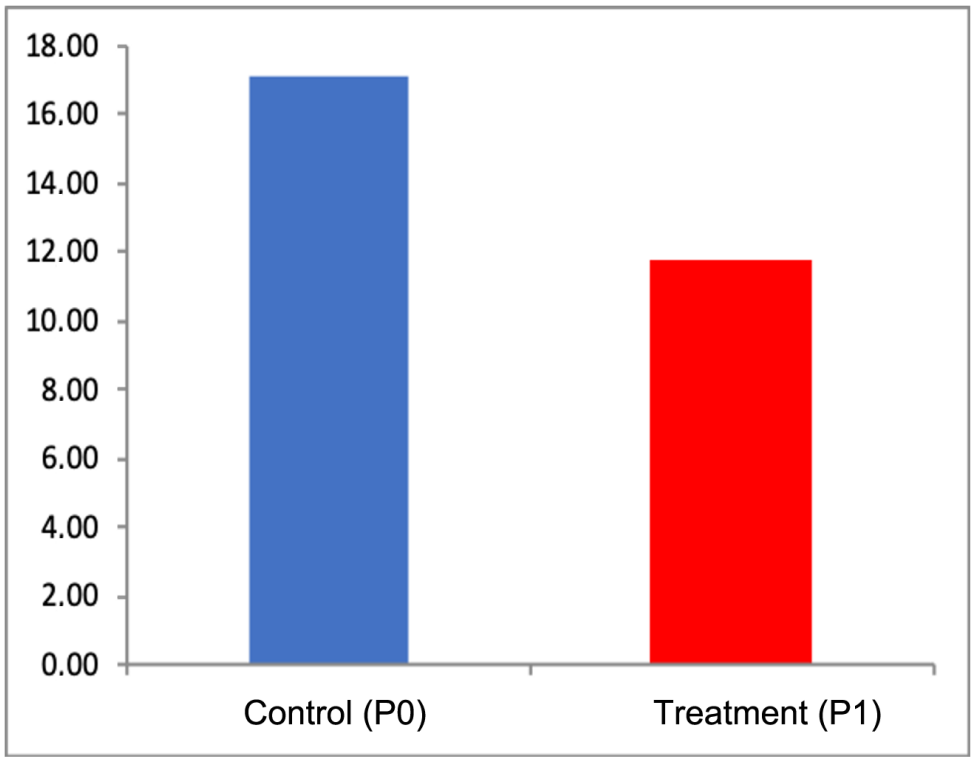

Figure 2.
Diagram of Difference in Average Spermatozoa Concentration between the Control Group (P0) and the Treatment Group (P1).

Table 1 and Diagram 3 show that the average Spermatozoa Concentration of the control group (P0) was 17,100±2.1828 million/ml and the Spermatozoa Concentration of the treatment group (P1) was 11.722±1.9157 million/ml. Based on the results of the analysis with *the t-independent test*, it was shown that there was a significant difference in the average Spermatozoa Concentration between the two groups *($p<0.05$)*. Figure 5.1 shows with blue arrows the spermatozoa in the counting chamber in the Treatment group were fewer than in the Control group.

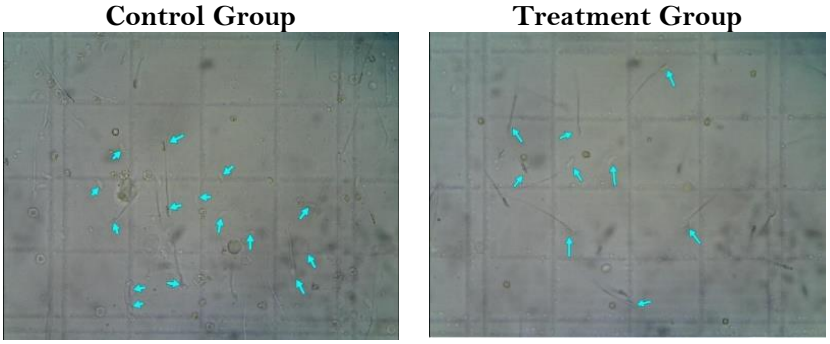

Figure 3.
Spermatozoa Concentration in the Counting Room (Total Magnification 400X).

The comparability analysis aimed to determine the comparison of Spermatozoa Motility between the control group (P0) and the treatment group (P1). The results of the significance analysis with the t-independent test are presented in Table 2.

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Table 2.
Difference in Average Spermatozoa Motility between the Control Group (P0) and the Treatment Group (P1).

Group	*n*	Rerata ±SB (%)	*p*
Control (P0)	18	66.089± 4.3566	0.000
Treatment (P1)	18	16.311 ± 3.9995	
Description: *n* = amount of data SB = Baku junction *p* = significance			

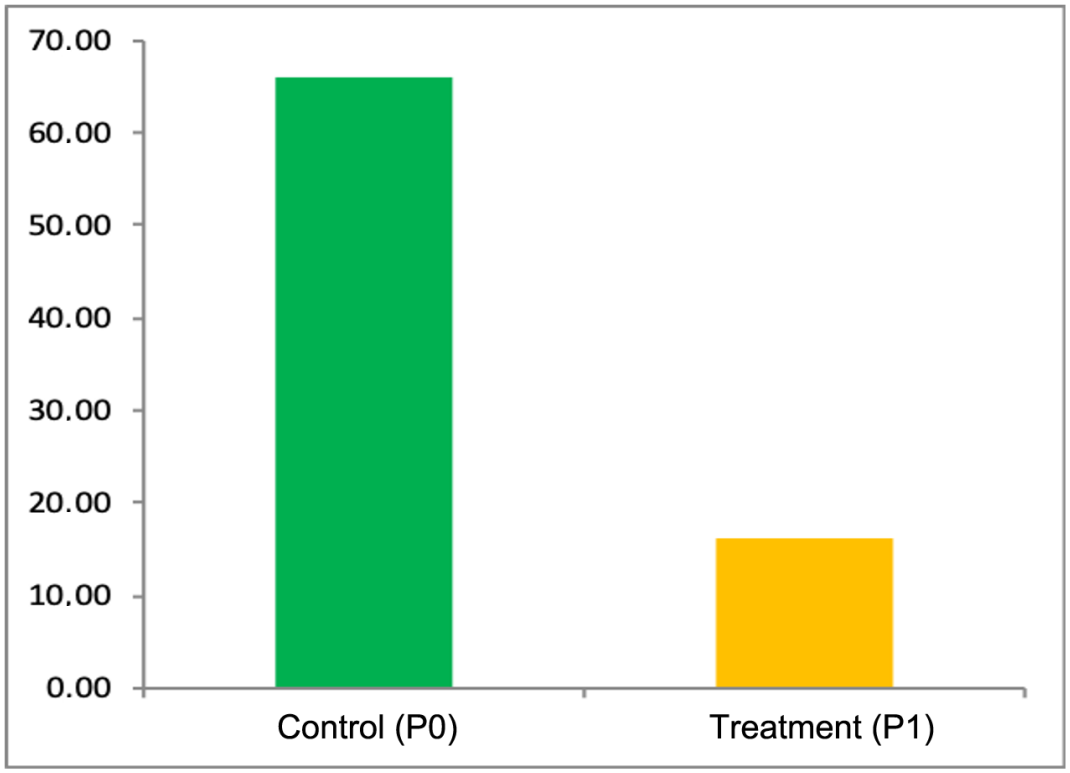

Figure 4.
Diagram of Difference in Average Spermatozoa Motility between the Control Group (P0) and the Treatment Group (P1).

Table 2 and figure 4 show that the average Spermatozoa Motility of the control group (P0) was 66.089±4.3566% and the Motility of the treatment group (P1) was 16.311±3.9995%. Based on the results of the analysis with *the t-independent test*, it was shown that there was a significant difference in the average motility of spermatozoa between the two groups *(p<0.05)*.

The comparability analysis aimed to determine the comparison of Spermatozoa Viability between the control group (P0) and the treatment group (P1). The results of the significance analysis with the t-independent test are presented in Table 3.

Table 3.
Differences in Average Spermatozoa Viability between the Control Group (P0) and the Treatment Group (P1).

Group	*n*	Rerata ±SB (%)	*p*
Control (P0)	18	77.700± 5.1758	0.000
Treatment (P1)	18	26.433 ± 5.4989	
Description: *n* = amount of data SB = Baku junction *p* = significance			

Table 3 and figure 5 show that the average Spermatozoa Viability of the control group (P0) was 77.700± 5.1758% and the Spermatozoa Viability of the treatment group (P1) was 26.433 ± 5.4989%.

Based on the results of the analysis with *the t-independent test*, it was shown that there was a significant difference in the average viability of Spermatozoa between the two groups *($p<0.05$)*.

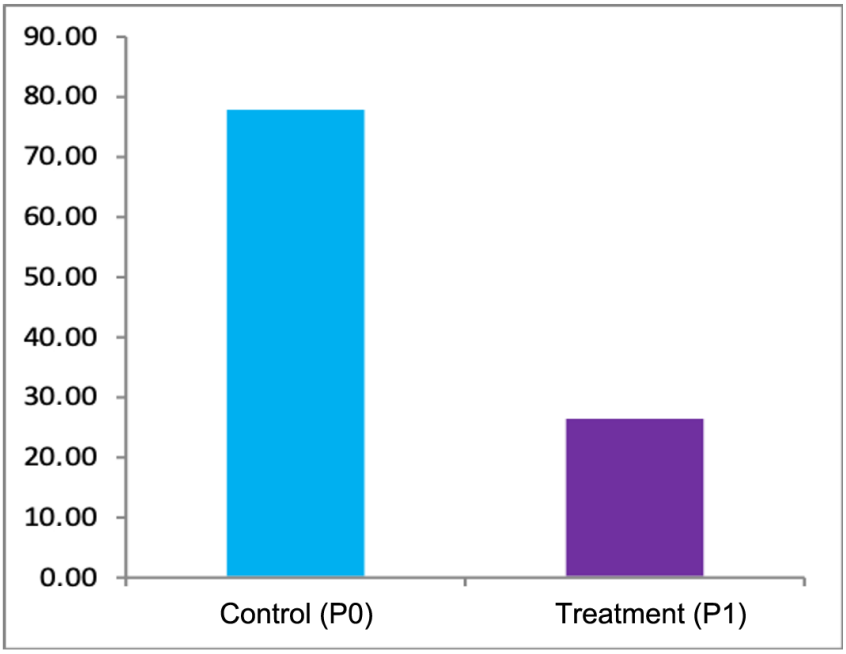

Figure 5.
Diagram of the Difference in Average Viability of Spermatozoa between the Control Group (P0) and the Treatment Group (P1).

Figure 6 shows the removal with Eosin-Nigrosin staining shows that the white viable spermatozoa (green arrow) did not absorb more Eosin in the control group than in the treatment group where the non-viable spermatozoa absorbed Eosin so that the spermatozoa heads were red and pink.

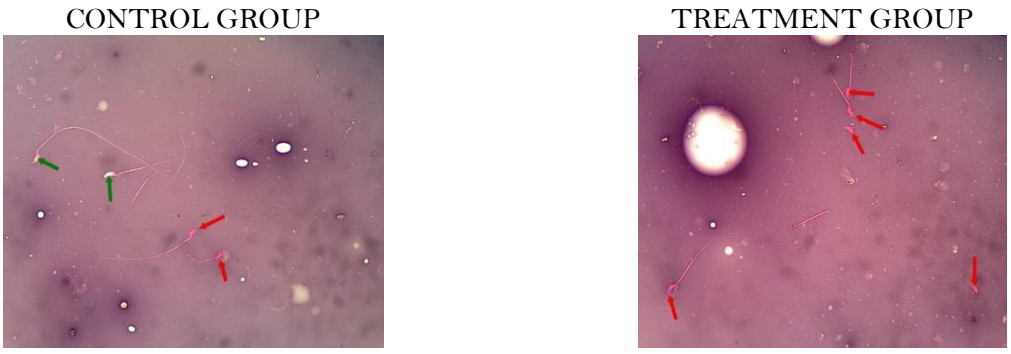

Figure 6.
Viability of Spermatozoa in Eosin-Negrosin Staining Sperm Removal (Total Magnification 400X).
Remarks: It appears that the spermatozoa are stained with Eosin-Negrosin staining, the head of the spermatozoa is pink, indicating the absence of viability (red arrow). The head of a spermatozoa without a pink red color (green arrow) indicates that the spermatozoa are still viable. It appears that in the treatment group many spermatozoa are not viable compared to the control group.

The comparability analysis aimed to determine the comparison of the Normal Morphology of Spermatozoa between the control group (P0) and the treatment group (P1). The results of the significance analysis with the t-independent test are presented in Table 4.

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Table 4.
Difference in Average Normal Morphology of Spermatozoa between the Control Group (P0) and the Treatment Group (P1).

Group	*n*	Rerata ±SB (%)	*p*
Control (P0)	18	82.078 ± 5.6769	0.000
Treatment (P1)	18	27.039 ± 5.1207	

Description: *n* = amount of data SB = Baku junction *p* = significance

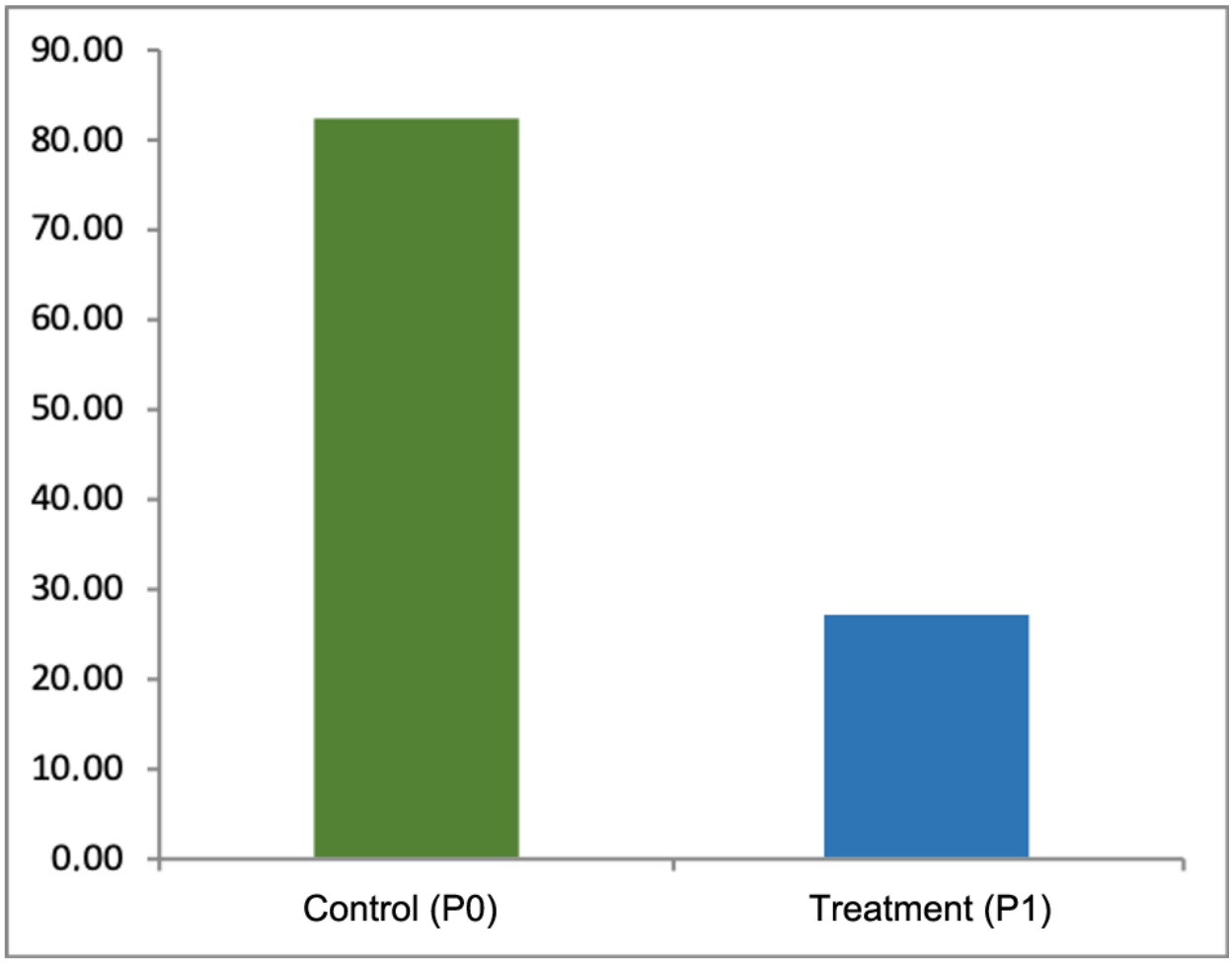

Figure 7.
Diagram of Average Difference in Normal Morphology of Spermatozoa between the Control Group (P0) and the Treatment Group (P1).

Table 4 and Figure 7 show that the average Normal Morphology of Spermatozoa of the control group (P0) was 82.078±5.6769% and the normal morphology of the treatment group of spermatozoa (P1) was 27.039 ± 5.1207%. Based on the results of the analysis with *the t-independent test*, it was shown that there was a significant difference in the average normal morphology of spermatozoa between the two groups *(p<0.05)*.

Figure 8 shows the morphological results of spermatozoa with Giemsa painting on sperm removal using a total magnification of 400X. Spermatozoa with normal spermatozoa head morphology black arrows (such as hooks and straight and not crooked tails) and abnormal spermatozoa morphology green arrows (crooked tail) and amorphous heads (purple arrows) appeared. It was seen in the treatment group that many spermatozoa with abnormal morphology were compared to the control group.

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CONTROL GROUP TREATMENT GROUP

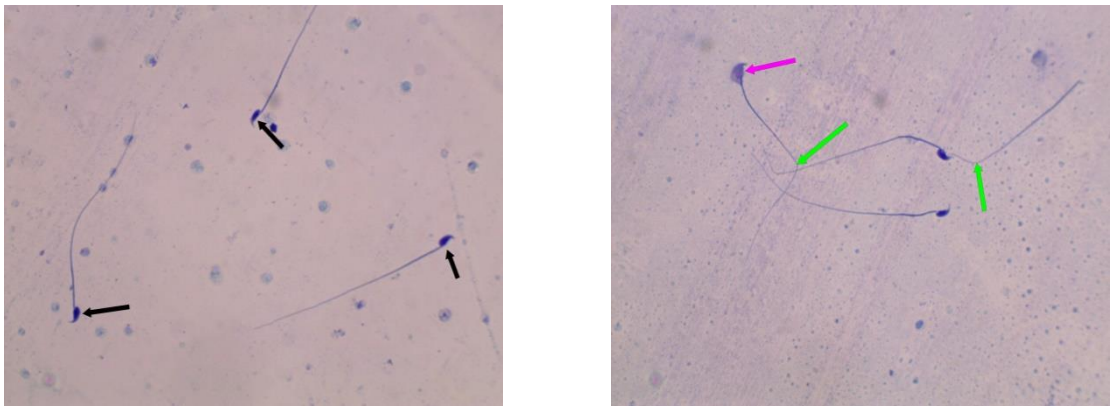

Figure 8.
Morphology of Spermatozoa in Giemsa Staining Removal (400X Total Magnification).
Description: It appears that spermatozoa are colored by Giemsa coloration, spermatozoa heads are normally shaped with black arrows (such as hooks and tails are straight and not bent) and abnormal spermatozoa morphology green arrows (crooked tails) and amorphous heads (purple arrows). Seen in the Treatment group Many spermatozoa with abnormal morphology compared to the Control group.

4. Discussion

In this study, using a purely experimental research design using a post-test only control group design [8] based on calculations with Busman and Sutyarso [9] as many as 36 mice (Mus musculus) with the Swiss Webster strain, aged 2-3 months, weighing 20-30 grams and healthy were included in the study. The sample was divided into 2 groups, namely the control group (P0) consisting of 18 male mice given Tween 3% as much as 0.5 ml and the treatment group (P1) consisting of 18 male mice given ethanol extract of Young Papaya Seeds (Carica papaya L.) 0.21 mg/gram BB as much as 0.5 ml for 36 days orally. Rats have physiological conditions that resemble humans [10].

The results of this study were given Ethanol Extract of Young Papaya Seeds (Carica papaya L.) In young adult mice (Mus musculus), there was an effect on decreasing spermatozoa concentration. The spermatozoa concentration in this study was obtained as an average result of the spermatozoa concentration in the treatment group being lower than in the control group. Statistical tests showed significant results on the difference in the average result of spermatozoa concentration in the two groups. This is suspected because the alkaloids, triterpenoids and steroids compounds contained in Papaya seeds (*Carica papaya* L.) are predicted to have cytosic effects on germinal cells and spermatogenic cells. In addition, the chemical compounds contained in Papaya seeds result in impaired cell metabolism which is an effect of the cytotoxic properties of secondary metabolites of alkaloids, triterpenoids and steroids that can reduce the degeneration of spermatozoa cells. Alkaloids and steroids are estrogenic in nature which results in a decrease in testosterone hormone by inhibiting the gonadotropin glands (GnRH) to inhibit the secretion of LH and FSH in the anterior pituitary. Due to the inhibition of LH hormone, the formation of testosterone hormone in Leydig cells is disrupted, as a result of which the spermatogenesis process will be inhibited, this will result in decreased spermatozoa concentration.

The results of this study are in accordance with previous research conducted by Wiryawan, et al. [4] where the results were obtained that there was a decrease in spermatozoa concentration in mice given oral Papaya Seed extract. The results of this study are in line with the research conducted by Airaodion, et al. [11] reported a significant decrease in spermatozoa concentration in wistar rats given ethanol extract of Papaya Seeds (*Carica papaya* L.). This is suspected to be caused by the content in Papaya Seeds (*Carica papaya* L.) resulting in disturbances in the biosynthesis of steroid hormones and disruptions of the spermatogenesis process which will affect spermatozoa concentration [11].

Secondary metabolites of flavonoids, alkaloids, saponins, are phytoestrogen compounds that are compounds similar to estrogen receptors [12]. The flavonoid content contained in Papaya Seeds (*Carica*

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papaya L.) is predicted to be as phytoestrogens. This research is in line with what was conducted by Nita, et al. [13] using *a literature review*, namely the effect of soy administration on the reproductive system. Where flavonoids, namely isflavones or genestein found in soybeans, can be phytoestrogens that can resemble estrogen receptors with the chemical structure of estradiol-17β. This structure can inhibit the enzyme 17β- hydroxysteroidoreductase is an enzyme needed to convert androstenadione into testosterone which can result in decreased levels of the hormone testosterone. As a result of high estrogen hormones, negative feedback from the hypothalamus-pituitary-testicles will decrease the levels of the hormone terstosterone as the largest androgen produced by the testes. Phytoestrogens themselves have a mechanism of interacting with ER, namely ERβ which has a higher affinity than ERα. Phytoestrogens can increase estrogen concentrations by binding to and inactivating the enzymes P450 aromatase, 5α-reductase, 17β-hydroxysteroidorereductase and tyrosine kinase which can have negative effects on the hypothalamus-pituitary-testicular so that spermatozoa quality decreases.

Research by Wijayanti, et al. [14] also compared methanol extract of old papaya seeds and methanol extract of young papaya seeds (Carica Papaya Lin.) to the quality and quantity of spermatozoa of male rats (Rattus nervegicus). It is reported that methanol extract of young papaya seeds has better quality and quantity of spermatozoa than old papaya seed extract [14]. This research is in line with the results of research by Ledoh and Irianto [15] who reported that the administration of papaya seed extract (Carica papaya L.) can reduce sperm quality. (Satriyasa & Pangkahila, 2010) found that 'Hexane Fraction and Methanol Fraction of Young Papaya Seed Extract Can Inhibit Spermatids of Male Mice (Mus musculus). With the inhibition of spermatids, the formation of spermatozoa will be disturbed, with the disruption of spermatozoa formation causing the quality of spermatozoa to also be disturbed. Busman and Sutyarso [9] reported that there was a significant decrease in spermatozoa quality, namely spermatozoa concentration, motility, and viability in mice given rhizome extract of teki grass orally for 35 days. caused by flavonoid compounds contained in teki grass being estrogenic or phytoestrogen can occupy estrogen receptors that function to suppress the production of FSH and LH where the FSH hormone functions as a stimulation of sertoli cells to secrete *Androgen Binding Protein* (ABP) to bind to testosterone. If there is an inhibition in ABP produced by sertoli cells, testosterone transport is also disrupted. Due to the inhibition of FSH and LH secretion due to the flavonoid compounds contained, the decrease in the amount of spermatogenesis produced by this spermatogenesis activity is inhibited. The flavonoid compounds contained in Papaya Seeds (*Carica papaya* L.) are thought to have the same mechanism in lowering spermatozoa concentration.

The results of this study found that there was an influence on the decrease in spermatozoa motility. Spermatozoa motility in this study was obtained with average results on progressive spermatozoa motility in the treatment group lower than in the control group. Statistical tests showed significant results on the difference in the average spermatozoa motility results in the two groups. This can happen due to the phytochemical content of flavonoids, tannins and alkaloids in Papaya Seed Extract (*Carica papaya* L). The flavonoid content can cause the spermatogenesis process to be disrupted where the properties of flavonoids are estrogenic which can form estrogen in the body increases. The effect of this increased estrogen will cause negative feedback to the hypothalamic-pituitary-testicular axis which leads to a decrease in the secretion of FSH and LH by the anterior pituitary so that the formation of the testosterone hormone produced by Leydig cells decreases so that the process of spermatogenesis and spermiogenesis will be disrupted. Disruption of the spermatogenesis process causes the quality of spermatozoa to be disturbed as well, especially impaired spermatozoa motility. In addition, tannin compounds found in the phytochemicals of Papaya Seeds (*Carica papaya* L.) are predicted to cause enzymes used when the release of energy in spermatozoa is inhibited by clumping proteins which are energy sources when the motility process occurs in spermatozoa. The protein is a constituent of microtubules on the outside, this protein can hydrolyze ATP which is used for the process of spermatozoa motility. Tannin content can result in *Reactive Oxygen Species* (ROS) which causes a decrease in the production of A TP in cell mitochondria, resulting in impaired spermatozoa motility.

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The alkaloid content in papaya seeds can cause a decrease in spermatozoa motility due to the disruption of the spermatogenesis process. Normally, this process begins with type A spermatogonia undergoing mitosis so that there is stem cell renewal and type B spermatogonia that continue to differentiate. Subsequently, type B spermatogonia form haploid round spermatids through the process of miosis. Spermiogenesis will occur the formation of condensation, nucleus acrosome and cellular reorganization which includes the development of the spermatozoa's tail. Sertoli cells mediate the spermiciation process i.e. cytoplasm is removed from the spermatid and mature sperm is released into the lumen of the seminiferous tubules, this process is also influenced by the regulation of endocrine hormones. The content of this alkaloid can interfere with this process because it is estrogenic which can interfere with the spermiogenesis process so that spermatozoa motility is disturbed (Wiryawan et al., 2015).

Spermatozoa motility comes from the movement of pushing spermatozoa on a part of the tail that resembles a whip. Motility is needed by spermatozoa to reach the ovum and penetrate in the fertilization process [4]. Physiologically, the process of spermatozoa motility occurs due to the *signaling pathway* process in the spermatozoa transmembrane through the receptor (R) which will affect VADC3. Dissolved guayl cyclase (sGC) will be activated by *Nitric oxide* (NO) thereby increasing *cyclic guanine monophosphate* (cGMP) which then activates potassium channels resulting in membrane potentials that stimulate the canals, ions and also the enzyme transmembrane *adenyl cyclase* (mAC). The *enzyme adenyl cyclase* can be activated by the bircarbonate (HCO3-) ion so that there is an increase in the concentration of cAMP and then mephosphorylation of protein kinase A (PKA) and protein tyrosine kinase (PTK) enzymes. The work of these enzymes then phosphorylates proteins in the flagellum of spermatozoa. Because the increase in Ca2+ in intracellular results in the movement of the flagellum which is chemotaxis during the process of capacitation of spermatozoa to the ovum. The content of the papain enzyme contained in papaya can result in toxicity in protein bonds and ion channels so that there is an obstacle to the influx of Ca2+ and Na+ into cells which causes differentiation in spermatozoa cells to be inhibited and spermatozoa motility will be disturbed. Research conducted by Rinaldi and Mujianto [8] by giving ethanol extract of Papaya Seeds (*Carica papaya* L.) orally for 36 days against copulation and the number of mice seedlings, this is due to the alkaloid compounds in Papaya Seeds can be toxic where the alkaloids will interfere with the activity of the ATP-ase enzyme on the cell membrane of spermatozoa, namely *the middle piece* or the middle part to produce ATP energy. As a result of ATP not being formed, it will result in dinein proteins and the Na+ and K+ pumps not functioning properly so that spermatozoa motility will be disturbed which will affect the results of copulation and the number of mice.

In the results of this study by giving Papaya Seed Ethanol Extract (*Carica papaya* L.) in young adult mice (*Mus musculus*) there was an effect on the decrease in spermatozoa viability. The viability of spermatozoa in this study was obtained as an average result of the viability of spermatozoa in the treatment group was lower than the control group. Statistical tests showed significant results on the difference in the average results of spermatozoa viability in the two groups [1] reported that there was a significant decrease in spermatozoa viability in male white rats given papaya leaf extract (*Carica papaya* L.) orally for 21 days due to flavonoid compounds contained in Papaya Seeds resulting in an increase in antioxidants in the body resulting in ROS. The ROS that is formed results in damage to the cell membrane of spermatozoa so that the integrity of the spermatozoa is disturbed which can cause damage to the spermatozoa's head so that it absorbs the color that indicates the spermatozoa is dead.

The viability of spermatozoa is the viability of spermatozoa. Viability can be correlated with motility which can be ensured by the strength of the plasma membrane of the spermatozoa which is the integrity of the spermatozoa [16]. Normal spermatozoa will look clear white under the microscope because the plasma membrane of the spermatozoa is still protected by lipoproteins. According to research by Busman and Sutyarso [9]. The flavonoid content in the rhizome of teki grass can reduce the viability of spermatozoa due to flavonoid compounds causing inhibition of GnRH secretion of FSH and LH so that the production of testosterone hormones decreases, where flavonoids have an estrogenic

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effect that causes negative feeds to the hypothalamic-pituitary-testicular axis. It is known that the function of the hormone testosterone functions to help the maturation of spermatozoa in the *epididymis* and the maintenance of seminiferous tubules in the process of spermatogenesis and spermiogenesis. If the testosterone hormone decreases, the secretion of necessary substances such as ions, substrates (glycogen, lactic acid, phospolipids and proteins), and enzymes (HDL, acid phosphotase and alkaline phosphotase) in the epididymis will be reduced so that spermatozoa will not obtain energy, enzymes and nutrients so that the integrity of the plasma membrane of the cell is damaged and causes the spermatozoa to die. This research is also supported by other research conducted by Prastika, et al. [16] by administering guava leaf ethanol extract (*Psidium Guajaya* L.) to antivertility activity in the testicles of mice (*Mus Muculus*) which resulted in a decrease in the viability of spermatozoa due to the content of flavonoids which are phytoestrogens causing negative feedback to the hypothalamus causing FSH and LH hormone levels to decrease and followed by a decrease in testosterone hormones. Reduced production of the hormone testosterone causes the spermatogenesis process to be inhibited so that there is a decrease in the viability of spermatozoa in the epididymis. The content of flavonoids, saponins, and tripertinoids in papaya leaves (*Carica papaya* L.) is predicted to have the same effect in reducing spermatozoa viability results. The study conducted by Nita, et al. [13] aimed to evaluate the antifertility effects of total flavonoids taken from *P. oleracea* L. administered orally in female rats. Flavonoid compounds can cause antifertility in female rats at a dose of 500 mg/kg BB with the results of quantitative analysis obtained that the total flavonoid content taken from *P. oleracea* L. is 339.21 µg/ml.

The results of viability observations carried out in this study using 1% Eosin staining, then examination under a microscope were obtained that the spermatozoa head did not absorb the red color, Eosin ate the integrity of the spermatozoa, was good and looked clear, and vice versa, the spermatozoa head that absorbed the color was declared dead spermatozoa. The following are the results of the viability of mouse spermatozoa (*Mus musculus*) in this study under a microscope seen in the image

In the results of this study by giving Papaya Seed Ethanol Extract (*Carica papaya* L.) in young adult mice (*Mus musculus*) there was an effect on the decrease in spermatozoa morphology. The morphology of spermatozoa in this study was obtained with average results on spermatozoa morphology in the treatment group which was lower than the control group. Statistical tests showed significant results on the difference in the average results of spermatozoa morphology in the two groups. This decrease in morphology is caused by secondary metabolite compounds, namely flavonoids contained in ethanol extract of young papaya seeds. Flavonoids can cause the formation of ROS, because the imbalance of ROS with these antioxidants will cause abnormal morphology of spermatozoa. ROS will damage the plasma membrane by apoptosis which causes the DNA structure of the cell to be damaged. If consuming antioxidants excessively, it will cause oxidative stress where the pro-oxidant function will be replaced by antioxidants, with high antioxidants can disrupt the balance of ROS and neutralization. In addition, flavonoid compounds also play a role in inhibiting the aromatase enzyme which is an enzyme that catalyzes the testosterone hormone into the estrogen hormone thereby increasing testosterone hormone levels which cause negative feedback to the pituitary hormones FSH and LH to decrease and cause testosterone hormone to decrease. This decrease in testosterone hormone levels leads to the formation of abnormal spermatozoa. This primary abnormal morphology is caused by a decrease in testosterone hormone which causes the protein α tubules which will be the basic components of microtubules and microfilaments in the process of spermiogenesis where the cytoplasm will go towards the back of the flagella. Secondary abnormalities are caused due to the disruption of the maturation process in the epididymis due to a decrease in testosterone hormone [17].

This research is in line with research conducted by Airaodion, et al. [11] using papaya seeds (*Carica papaya* L.) can increase spermatozoa abnormalities (spermatozoa morphology) in Wistar mice due to the content of papain and *chymopapain* enzymes in papaya leaves which can hydrolyze semen proteins which will cause damage to spermatozoa cells. The enzymes papain and *chymopapain* can damage Sertoli cells

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so that the formation and maturation of spermatogenesis can be disrupted so that abnormal morphological abnormalities occur in spermatozoan cells. In addition, it is also supported by the research of Hatify, et al. [18] using papaya leaf extract (*Carica papaya* L.) can cause spermatozoa abnormalities in mice caused by the papain enzyme which can cause protein synthesis to be disrupted where the synthesis of polypeptides and dipeptides decreases. Sertoli cell damage is also caused by the odor of the papain enzyme and the degenerated seminiferous tubules.

The research conducted by Nita, et al. [13] Extract and fraction of methanol of Javanese Date Fruit (*Phoenix Dactylifera*) fruit water revealed the morphology of spermatozoa in male rats due to flavonoid compounds causing ROS which can interfere with the spermatogenesis process that damages macromolecules such as lipids, proteins and nucleic acids. This result is in accordance with *the antioxidant* screening found in papaya seeds (*Carica papaya* L.) and the *results of antioxidants* are very strong with IC50 18.9506. Antioxidants are closely related to ROS buildup. If the production of ROS in the body is not controlled, it results in oxidative stress which can result in damage to spermatozoa DNA, decreased spermatozoa function, and damage to spermatozoa membranes. But on the other hand, antioxidants can help and support the process of spermatogenesis, refraction capacity and sperm function with oocytes if the production of ROS in the body decreases. According to research conducted by Kang, et al. [10] it can increase spermatozoa motility after being given avocado juice given exposure to cigarette smoke. This is due to the flavonoid content in avocados as antioxidants that can neutralize free radicals. In this study, there are several limitations, first, it has not been studied about the molecular pathways that affect the effects of entifertility such as the effect on the expression of AR receptors in mouse testicular cells.

5. Conclusion

The results of this study support the theory that administering ethanol extract of young papaya seeds (Carica papaya L.) to young adult mice (Mus musculus) led to a decrease in spermatozoa quality in the treatment group compared to the control group. This is evidenced by several key findings, namely lower spermatozoa concentrations in the treatment group, rapid progressive spermatozoa motility, and lower normal spermatozoa morphology and viability compared to the control group.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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