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Original Article

Effect of pH and neutrophil count on the motility and persistence of spermatozoa in the vagina of candidiasis rat models

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Abstract

Sexual violence is a global issue affecting individuals regardless of their relationship to the perpetrator or the setting. Microscopic examination of spermatozoa from vaginal swabs is crucial in investigating cases of sexual intercourse to determine the time of the crime. Factors such as vaginal pH and neutrophil count influence the motility and persistence of spermatozoa in the vagina, particularly in conditions like candidiasis, highlighting the need for further research in this area. This study aimed to determine the effect of pH and neutrophil count on the motility and persistence of spermatozoa in the vagina with candidiasis. An experimental study was conducted using white rats (*Rattus norvegicus*) of the Wistar strain, with four male rats providing spermatozoa samples and 32 female rats receiving treatment. The female rats were divided into two groups: the normal group and the candidiasis model group. In both groups, the female rats were given vaginal insemination of spermatozoa. Variables measured included pH, neutrophil count, motility, and persistence of spermatozoa in the vagina. Data were analyzed using the Mann-Whitney test, followed by the Spearman correlation test. The findings revealed that spermatozoa motility lasted up to three minutes in normal rats, whereas in the candidiasis model, it was reduced to two minutes. Additionally, spermatozoa persistence in the vagina lasted up to six days in the normal group compared to up to three days in the candidiasis model. There were significant differences in pH, neutrophil count, motility, and persistence of spermatozoa in the vagina between the normal group and the candidiasis model (all had $p<0.001$). There was a correlation between pH and neutrophil count with the motility and persistence of spermatozoa in the rat's vagina ($p<0.001$). In conclusion, vaginal pH and neutrophil count influence the motility and persistence of spermatozoa in the vagina of candidiasis rat models.

Keywords: Vaginal candidiasis, vaginal pH, vaginal neutrophil count, motility of spermatozoa, the persistence of spermatozoa

Introduction

Sexual violence is any sexual act, attempt to obtain a sexual act, or other act directed against a person's sexuality using force by anyone, regardless of their relationship to the victim, in any

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setting [1,2]. Sexual violence is a form of violation of decency which is not only a national legal problem in a country but has become a legal problem in all countries in the world or a global problem [2,3]. The World Health Organization (WHO) reported that more than a quarter of women aged 15–49 years who have been in a relationship have experienced physical and/or sexual violence by an intimate partner at least once in their lifetime (since the age of 15) [4]. WHO also estimated the prevalence of intimate partner violence ranged from 20% in the Western Pacific region, 22% in high-income countries and Europe, 25% in the Americas, 33% in the African region, 31% in the Eastern Mediterranean region, and 33% in Southeast Asia region in 2018 [4].

Based on the 2023 Annual Record Sheet released by the Indonesian National Commission for Anti-Violence against Women, there were 4,322 cases of sexual violence against women in the personal sphere with the most cases being sexual intercourse with 845 cases [5]. Many cases of sexual violence also occur among children, where, based on data from the Indonesian Child Protection Commission in 2023, violence against children was dominated by sexual violence/crimes; one of the most frequent cases was sexual intercourse [6].

In proving cases of sexual intercourse, it is necessary to carry out microscopic examination of spermatozoa from vaginal swabs [7,8]. This examination is useful to see whether there are spermatozoa either still moving (motile) or immotile/persistent [7]. This will also help in determining the time when the crime of sexual intercourse occurred [7,8]. Several factors influence the motility and persistence of spermatozoa in the vagina, such as vaginal pH, the role of spermatozoa phagocytes by polymorphonuclear (PMN) neutrophil cells, and the vaginal microbiome [9,10].

Cases of sexual violence in the form of sexual intercourse can occur in all age ranges, especially in reproductive age [4,5]. It is not uncommon for victims of sexual violence to also occur in women who are experiencing vaginal discharge, especially candidiasis, where it is estimated that 75–80% of all women suffer from vulvovaginal candidiasis infection at least once, and this infection accounts for more than 25% of all infectious vaginitis [11,12]. In candidiasis, there is a change in the microbiome profile which results in an increase or decrease of vaginal pH, as well as an increase in neutrophil count in the vagina as the body's defense mechanism against infection through several mechanisms, including phagocytosis, extracellular degranulation, neutrophil extracellular trap (NET) and trogocytosis [13-15].

The condition of vaginal discharge due to candidiasis causes vaginal pH changes that will affect the motility and persistence of spermatozoa in the vagina [16-18]. Apart from that, the body's defense in the vagina, which is played by PMNs, will respond to spermatozoa and other pathogens such as *Candida albicans* (*C. albicans*) in the vagina, which can affect the motility and persistence of spermatozoa [19,20]. Until now, the average change in pH or neutrophil count in the vagina in candidiasis is unknown. It is also unknown how long the motility and persistence of spermatozoa in the vagina with candidiasis and its relation to changes in pH and the number of neutrophils in the vagina. Conducting research with human subjects can be challenging due to ethical concerns and the difficulty in recruiting participants. Therefore, the aim of this study was to determine the effect of pH and neutrophil count on the motility and persistence of spermatozoa in the vagina of candidiasis rats.

Methods

Study design and setting

An experimental study using animal models was conducted at the Animal Research Facilities (ARF) Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. This study obtained male and female white rats (*Rattus norvegicus*) of the Wistar strain from PT. Bio Farma, Bandung, Indonesia. *C. albicans* cultures were sourced from candidiasis vaginalis patients acquired from the Parasitology Laboratory, Faculty of Medicine, Universitas Indonesia.

Selection was carried out using a simple random sampling technique. A minimum sample size of 32 female rats was required based on the Federer formula. The study involved two experimental groups: a normal female rat group and a female rat with candidiasis model, with

each group consisting of 16 female rats. For sperm insemination, four male rats were used (sperm samples for one rat were inseminated into the vaginas of four female rats).

Inclusion and exclusion criteria

The rats included in this study were aged 8–10 weeks, weighing 200–250 grams, healthy, had no wounds or anatomical abnormalities, and had been acclimatized. The criteria for male rats' sperm to be inseminated into female rats' vaginas included a pH range of 7.2–7.8, sperm concentration of over 35 million/mL ejaculate, more than 3.9% normal sperm morphology, total sperm motility of more than 40% with progressive motility exceeding 29%, non-progressive motility at 1%, and immotile sperm at less than 19%. Rats that were sick or died during adaptation or treatment and male rats with asthenozoospermia were excluded from the study. Female rats that died during regular vaginal swab sampling preventing the collection of complete serial data until the end of the study, were dropped out of the study.

Animal preparation and acclimatization

The rats were acclimatized for one week and given Ratbio feed (which contains 60% carbohydrates, 20% protein, 4% fat, 4% crude fiber, 12% calcium, and 0.7% phosphorus), water *ad libitum* and put in a closed system cage to minimize exposure with various factors that can affect spermatozoa and vaginal group of the rats. The temperature and humidity were kept within a range of 23±10°C, under 12 hours dark and 12 hours light cycle.

Candidiasis rat models

The candidiasis rat group was given a subcutaneous injection of 0.5 mg estradiol valerate in 50 µL of sesame oil in the lower abdomen of female rats every day for five days before vaginal inoculation. For *C. albicans* inoculation, a 2.5×10^6 cell suspension (or desired inoculum in the range between 5×10^4 and 5×10^8) of *C. albicans* blastospores from stationary phase culture in 0.02 mL of sterile phosphate-buffered saline (PBS) was prepared. A total of 0.02 mL of yeast cell suspension in PBS was inoculated into the vagina using a micropipette and sterile tip. The tip was inserted into the end of the vaginal lumen, approximately 1.5 cm close to the cervix. The inoculation was performed under anesthesia to minimize rat distress. After 3–5 days, a vaginal swab was taken using sterile cotton swabs. The vaginal swab was applied to an object glass. After drying, the slide was stained with KOH 10% solution to confirm a *C. albicans* infection, characterized by the appearance of hyphae and spores from *C. albicans*.

Sperm collection and examination

Male rats were euthanized using a lethal dose of ketamine 80 mg/kg body weight (BW) (PT. Dexa Medica, Tangerang, Indonesia). Sperm samples were taken from the cauda epididymis of male rats. At that location, they were clamped and then cut. The sperm was removed from the cut part by squeezing it into a petri dish containing 1.5 mL of Vitrolife Sperm Rinse (GlobalMed Groups, Miami, USA) buffer media containing human serum albumin. Sperm obtained from male rats was confirmed to be present and not experiencing asthenozoospermia by examining motility, viability, and spermatozoa count before insemination.

After sperm collection from the male rats, pH analysis was conducted using the pH indicator MQuant Merck 1.09543.0001 (Merck, Darmstadt, Germany). The sperm underwent microscopic analysis to evaluate sperm count, motility, viability, and morphology. Sperm count was assessed using a sperm counting chamber Makler (016261 Makler counting chamber, Darmstadt, Germany), motility was directly observed under a microscope with 0.9% NaCl, and eosin staining was used to assess viability and morphology.

Evaluation of the estrous phase

Before insemination, it was ensured that the female rat was in the estrous phase. The macroscopic examination involved observing the vagina expected with less moist tissues and a light pink color, with more prominent striations. Microscopic examination was performed by obtaining vaginal discharge through vaginal lavage, fixing it using a 90% alcohol solution, and staining it with Giemsa. The estrus phase exhibited abundant anucleated cornified epithelial cells, with granular cytoplasm and irregularly shaped cells.

Insemination of sperm into the vagina of female rats

Using a micropipette with a tip at a 120° angle, the female rat was held in a supine position for insemination. A total of 200 µL sperm from a 1.5 mL sperm rinse in a petri dish was inserted into the vagina of each female rat to a depth of 1.5 cm. After insemination, the rats were kept in a supine position for up to one minute before returning them to their cages.

Vaginal pH measurement of female rats

An MQuant Merck 1.09543.0001 pH indicator was inserted with a small tip into the vagina of a female rat to a depth of 1.5 cm. The pH indicator was removed after ten seconds from the vagina and observed for color change according to the pH value of the indicator. Observations were conducted from pre-sperm insemination/day 0 (D0) through post-insemination day 6 (D6).

Vaginal swabs of female rats

A sterile cotton swab with a diameter of 3 mm and a length of 12 mm was moistened first using Vitrolife SpermRinse. The cotton swab was inserted into the vagina of the female rat to a depth of 1.5 cm and rotated the swab clockwise for 20 seconds at a 45° angle. The swab was applied to an object glass that had been previously labeled. Vaginal swabs were collected along with pH measurements. Vaginal swabs were collected before and after sperm insemination, from day 0 until day 6.

Examination of the motility of spermatozoa

The motility of spermatozoa was examined on an object glass that had been dropped with 0.9% NaCl, using an Olympus CX23 light microscope (Olympus, Tokyo, Japan) with 100× magnification. The motility of all spermatozoa within a defined area of the field was assessed to determine whether motile spermatozoa were still present (rapidly progressive, slowly progressive, or non-progressive) until immotile spermatozoa were observed. This examination was conducted as soon as possible. Observations were made from the start of the minute 1 (M1) until the minute 6 (M6) after insemination to determine how long the sperm was motile in the vaginal of female rats.

Examination of the persistence of spermatozoa

A cotton swab soaked in Vitrolife SpermRinse was wiped on an object glass and rotated at 360°. After drying, the slide was stained with Diff Quick Solution. First, the slide was soaked in methanol and eosin for 30 seconds and then rinsed with a phosphate buffer solution. Next, it was soaked in methylene blue for 30 seconds and then rinsed with water. Then, the samples were ready for the examination of the persistence of spermatozoa and neutrophil count. Examination of the persistence of spermatozoa was performed using a light microscope (Olympus CX23, Tokyo, Japan) with 400× magnification. The assessment focused on whether sperm was still present across the entire slide. The evaluation checked whether whole sperm cells or only parts such as the head, neck, or tail remained. Observations were made each day, from day 1 to day 6 after insemination, to determine how long the sperm remained detectable in the vaginal of female rats.

Examination of the neutrophil count

Using the same light microscope with 400× magnification, the neutrophil count was assessed. PMN neutrophils were counted in 10 different microscopic fields on the object glass slides. The average neutrophil count was calculated at different times before and after sperm insemination. Observations were made from day 0 to day 6 after sperm insemination.

Statistical analysis

To determine the differences in pH, neutrophils count, motility, and persistence of spermatozoa between groups, the Mann-Whitney test was used due to the data was not normally distributed. To determine the correlation between changes in pH and neutrophil count with the duration of motility and persistence of spermatozoa in the vaginal of female rats, the Spearman correlation test was used. All data were analyzed using SPSS version 27.00 for Macbook (SPSS Inc., Chicago, USA).

Results

Comparison of vaginal pH between normal and candidiasis model

Before sperm insemination (D0), the vaginal pH of the normal rat group leaned neutral to alkaline ranging from 7.6–8.0. However, in the candidiasis model was alkaline, ranging from 8.8–9.3. The average vaginal pH from day 0 until day 6 in the normal rat group ranged between 7.61–7.87, while in the candidiasis model group, it was between 8.20–8.51. Significant differences in vaginal pH (p<0.001) were observed between groups (**Table 1**). The vaginal pH in the normal group showed no meaningful changes from day 0 to day 6, but the candidiasis model showed a significant reduction from alkaline approximate to neutral (**Figure 1**).

Table 1. Comparison of pH, motility and persistence of spermatozoa and neutrophils count in the vagina between groups of rats in normal conditions and candidiasis models

Variables	Groups		p-value [a]
	Normal condition	Candidiasis models	
	Median (min-max)	Median (min-max)	
Vaginal pH	7.79 (7.61–7.87)	8.38 (8.20–8.51)	<0.001*
Motility of spermatozoa, minute	3 (2–3)	1 (1–2)	<0.001*
Persistence of spermatozoa, day	5 (4–5)	2 (1–3)	<0.001*
Vaginal neutrophils count	24.35 (21.00–27.86)	35.21 (32.71–37.14)	<0.001*

*Statistically significant at p=0.001
[a] Analyzed using the Mann-Whitey test

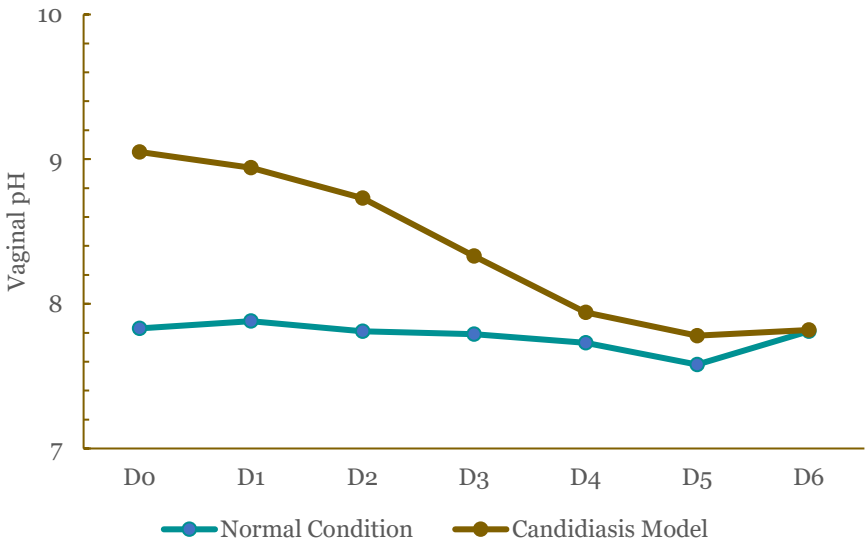

Figure 1. Mean vaginal pH of female rats before sperm insemination (D0) until six days after sperm insemination (D6).

Comparison of motility and persistence of spermatozoa between normal and candidiasis model

In the normal rat group, spermatozoa were observed to be motile in the vagina up to minute 3, whereas in the candidiasis model lasted up to minute 2 after sperm insemination. Notable differences were demonstrated among groups with p<0.001 (**Table 1**).

For the persistence of spermatozoa, in the normal rat group, spermatozoa could still be found in the vagina until day 5. Meanwhile, in the candidiasis model until day 3 after sperm insemination. Significant differences in the persistence of spermatozoa (p<0.001) were observed between the normal rat group and the candidiasis model (**Table 1**).

Comparison of neutrophil count between normal and candidiasis model

Before sperm insemination (D0), no neutrophils were detected in the vaginal samples of female rats in the normal rat group. However, in the candidiasis model group, the average vaginal neutrophil count ranged from 23 to 39. The vaginal neutrophil count exhibited significant

fluctuations from D0 to D6 in both the normal and candidiasis model groups. There was an initial increase on day 1, followed by a decline until day 3, and then a subsequent rise from day 4 through day 6 (**Figure 2**). The average vaginal neutrophil count in the normal rat group ranged between 21.00–27.86, while in the candidiasis model group, it ranged from 32.71 to 37.14 (**Table 1**). **Figure 2** depicts an increase in the vaginal neutrophil count of female rats' vaginas following sperm insemination.

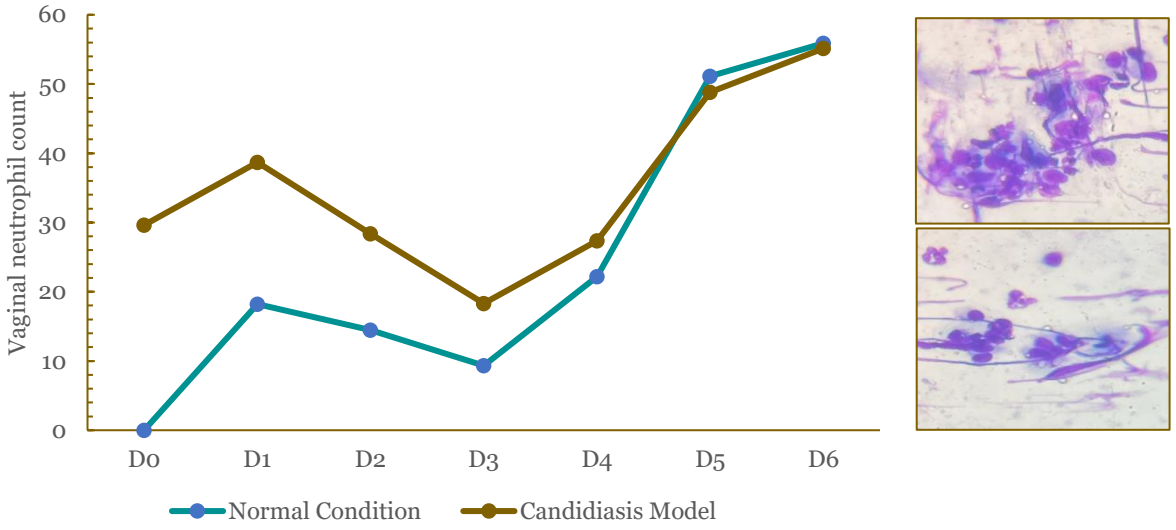

Figure 2. Mean vaginal neutrophil count of female rats before sperm insemination (D0) until six days after sperm insemination (D6). Legend: representative microscopic examination revealing an increase in neutrophil count in the vagina as immunity against spermatozoa and spores of *Candida albicans*.

Correlation between pH and neutrophil count of vagina with motility of spermatozoa

There is a moderate negative correlation between vaginal pH and the vaginal neutrophil count with the motility of spermatozoa, with r=-0.695 and r=-0.598, respectively, with both had p<0.001 (**Table 2**).

Table 2. Correlation between pH and neutrophil count of vagina with motility of spermatozoa

Variables	Motility of spermatozoa	
	r	*p*-value
Vaginal pH	-0.695	<0.001
Vaginal neutrophil count	-0.598	<0.001

Correlation between pH and neutrophil count of vagina with persistence of spermatozoa

It was found that vaginal pH and vaginal neutrophil count had very strong negative correlations with the persistence of spermatozoa in the female rats' vaginas with r=-0.820 and r=-0.803, respectively, and both had p<0.001 (**Table 3**).

Table 3. Correlation between pH and neutrophil count of vagina with persistence of spermatozoa

Variables	Persistence of spermatozoa	
	r	*p*-value
Vaginal pH	-0.820	<0.001
Vaginal neutrophil count	-0.803	<0.001

Discussion

There were differences in vaginal pH between groups of rats in the normal group and the candidiasis model. In the normal group of rats, vaginal pH tended to be neutral to alkaline, between 7.61–7.87. However, in the vagina of the group of rats with candidiasis models, the pH

was relatively high/alkaline compared to the group of rats in the normal group. In humans, *C. albicans* usually grow well in acidic pH, adjusting the vaginal pH between 3.8–4.5 [21,22]. However, in the candidiasis model that was formed, it produces an alkaline vaginal pH of more than 8.5 with a range from 8.8–9.3. The vaginal pH in humans tends to be acid due to the normal flora of *Lactobacillus* which produces lactic acid [23,24], whereas in rats there are no *Lactobacillus*. Various environmental groups can influence the morphology of *C. albicans*. Naturally, *C. albicans* is not only able to adapt to environmental pH but can also modulate extracellular pH, actively alkalizing the surrounding environment so that *Candida* can live in an alkaline environment in animal models of Candidiasis [16,25-27].

After sperm insemination, there was a change in the vaginal pH of rats both in the normal group and the candidiasis model. Under the normal group, the change in pH is not very significant from the first to the last observation in the pH range between 7.5–8 because the pH of spermatozoa is almost the same as the pH of the vaginal rats [9,27,28]. Meanwhile, in the candidiasis model rat group, there was a fairly large decrease from pH 9 since the model was created to pH 7.5. This is caused by candidiasis infection that only lasts until day three so that when the infection has improved, the vaginal pH returns to its normal pH. Insemination of sperm in the vagina does not significantly affect changes in pH in the vagina. The natural acidity group in the vagina determined by the microbiome greatly determine the pH in the vagina [23-25].

There were differences in neutrophil count in the vagina between groups of rats in the normal group and the candidiasis model. Under the normal group, there were no neutrophils found in the vaginal rats before sperm insemination, whereas, in the candidiasis model, there were neutrophils in the vagina. This is because vaginal infections of *C. albicans* promote the innate immune defense, especially involving cellular components, which is mainly carried out through the process of phagocytosis by neutrophils and macrophages [29-31]. The first step of the phagocytosis process involves macrophages and PMN neutrophil cells which recognize pathogen-associated molecular patterns (PAMPs) on *C. albicans* cell walls through pattern recognition receptors (PRRs) located on the phagocytic cell membrane, endosomes and cytoplasm. The binding of these receptors can phagocytose and destroy fungal cells in phagolysosomes through several oxidative and non-oxidative mechanisms, including the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), the expression of several antimicrobial peptides and the activity of hydrolytic enzymes. Fungi and bacteria induce the formation of neutrophil extracellular traps (NETs) through activated PMN neutrophils that capture and kill fungi and bacteria extracellularly [31,32].

After sperm insemination, there was a change in the vaginal neutrophil count of rats in both the normal group and the candidiasis model. The increase was quite high on day one after sperm insemination. The vaginal mucosa is characterized by several important components, including the presence of mucus secretions, low pH, vaginal discharge, and the presence of immune cells that function to kill spermatozoa [33,34]. PMN neutrophil cells are the first phagocytes to encounter spermatozoa in the female reproductive mucosa, and they rapidly reduce the motility (within five minutes) and viability (within 20 minutes) of spermatozoa to as low as 1% upon contact, but the mechanisms of spermicide are still not completely defined and controversial [10,35,36]. Although there is strong evidence for phagocytosis, it is unclear how PMN (with a diameter of 9 μm) can phagocytose much larger spermatozoa (with a head diameter of 5 μm and a length of 50 μm). The latest theory suggests that one of the mechanisms of neutrophil spermicidal effects is trogocytosis [10,36].

After day 1, the neutrophil count in the vagina of rats in both the normal group and the candidiasis model decreased until day 3, then began to increase again on days 4 to 6. This is because the rats have entered the metestrus and diestrus phases, resulting in a significant increase in the neutrophil count [37,38]. Furthermore, neutrophils are recruited in large numbers into the vaginal lumen to maintain constant neutrophil immune surveillance against pathogens and to control commensal microbiota balance before ovulation in the next estrus phase [38]. From microscopic examinations, it can be seen that there was an increase in neutrophil count related to the presence of spermatozoa entering the metestrus and diestrus cycles, identified by an even distribution of neutrophils in the preparation.

There were differences in the motility and persistence of spermatozoa between the vaginal rats in the normal group and the candidiasis models. The motility and persistence of spermatozoa were longer in the normal group compared to the candidiasis model. The spermatozoa are the only cells whose activity is outside the body of the male, and in the inconsistent chemical environment of spermatozoa, seminal plasma may have a major influence on spermatozoa quality and pH is one of the most important factors determining semen quality [9,39,40]. The motility, viability, capacitation and acrosome reaction functions of spermatozoa are highly dependent on pH. During passage in the male and female genital tract, spermatozoa cells exhibit proper regulation of proton gradients and thereby regulate intracellular pH [40,41].

In the candidiasis model, rats with alkaline pH caused a decrease in the motility and persistence of spermatozoa in the vagina. This is in accordance with several studies that showed spermatozoa function is distorted/disturbed at high/low pH, indicating the existence of a dynamic pH regulation system in spermatozoa [9,41]. The more acidic and alkaline the vaginal pH, the motility and persistence of spermatozoa will also decrease. Several studies related to the effect of pH on birds, fish, shellfish, and mammals have been carried out, and almost all of the results showed a very significant effect of pH on the motility, viability, and capacitation of spermatozoa [41,42].

The mechanism of pH regulation in mammalian spermatozoa is very complex [41]. The influx of HCO_3^- ions, voltage-gated proton channels (Hv1), and Na^+/H^+ exchanger (NHE) are three mechanisms for regulating spermatozoa pH. The pH greatly influences the durability of spermatozoa because it is closely related to the metabolism (energy use) of spermatozoa [9]. The energy required for motility is obtained from the intracellular supply of ATP. The energy directly used for the motility of spermatozoa is produced by the tail fibers coming from the description of ATP [9,41].

Studies on bovine spermatozoa have established the fact that intracellular pH substantially regulates spermatozoa motility. A decrease in pH from 6.5 to 6 corresponds with a linear decrease in spermatozoa motility, suggesting a potential role of pH in regulating spermatozoa motility [41,43]. A study on spermatozoa under controlled viscosity and temperature groups of reproductive tract fluids, with varying pH levels, found the changes in spermatozoa motility, highlighting the potential role of pH in regulating spermatozoa motility [41]. Kinematic studies examining the effect of pH on bovine spermatozoa function showed higher values of kinetic parameters, membrane integrity, and mitochondrial activity at pH 7 and 7.5, while pH below 6.5 and above eight resulted in impaired motility with a partial decrease in spermatozoa size [41,44].

Other factors influencing spermatozoa motility include the motility inhibiting factor (MIF II) and motility initiating protein (MIP), which are strongly affected by pH levels. Lower pH (4.0–4.5) inhibits spermatozoa motility, while optimal pH for MIF II activity is observed between 6.5 and 7.5; MIF II activity diminishes at alkaline pH levels above 7.5 [41,45]. Spermatozoa motility is enhanced when MIP reaches its maximum potential at pH 8, while neutral pH results in minimal MIP activity. Both inhibitory factors (MIF II) and initiating proteins (MIP) were pH-dependent activity [41,46].

In the normal group, the motility and persistence of spermatozoa in the vagina were longer compared with the candidiasis model. This is because spermatozoa that enter the vagina were considered foreign objects and the body's natural defense was played by neutrophils to carry out the process of phagocytosis, extracellular degranulation, and NET. Currently, it has been explained that apart from phagocytosis, neutrophils can also carry out trogocytosis to kill tumor cells [47] and unicellular flagellated parasites [48]. Trogocytosis is a NET-independent, contact-dependent, and serine protease-dependent mechanism, which acts over short time periods (minutes) and relies on PI3-kinase because it mediates the neutrophil engulfment process [47]. The trogocytosis mechanism is carried out in two stages. First, neutrophils retain spermatozoa by 'biting' most of it at the spermatozoa head, and rapidly reducing the stability of the spermatozoa's electrochemical membrane gradient. As a result, mitochondria reduce ATP production and cause a drastic reduction in spermatozoa motility; this avoids spreading and killing spermatozoa. Second, neutrophils then produce NETs to trap and eliminate spermatozoa with neutrophil granular secretions like elastase, neutrophil expressed (ELANE), myeloperoxidase (MPO), calprotectin, defensin, etc., to support spermatozoa elimination through vaginal fluids [47,48].

Through the formation of NETs, which are DNA structures released by neutrophils, spermatozoa cells are captured and their motility is restrained until they are phagocytosed by other neutrophils [49]. These sequential steps could be a rapid, low-impact way to regulate the ability of neutrophils to avoid damage to the vaginal mucosa [10,47-50].

This study demonstrated that both pH and the vaginal neutrophil count significantly impact the motility and persistence of spermatozoa. Each of these factors individually contributes to and collectively reduces the motility and persistence of spermatozoa, as illustrated in **Figure 3**. After inoculating *C. albicans* into the vagina until the candidiasis model was formed, there was a change in vaginal pH to more than 8.5 and an increase in the number of neutrophils in the vagina. Spermatozoa insemination also further increases the neutrophil count in the vagina. An increase in pH exceeding 8.5 inhibits HCO_3^- influx, voltage-gated proton channel (Hv1), and Na^+/H^+ exchanger activity, thereby impacting sperm mitochondria and reducing the motility and persistence of spermatozoa in the vagina. Additionally, an elevated neutrophil count promotes neutrophil degranulation, resulting in the production of ROS, phagocytosis, and neutrophil extracellular traps (NETosis), which also contribute to the decreased motility and persistence of spermatozoa in the vagina.

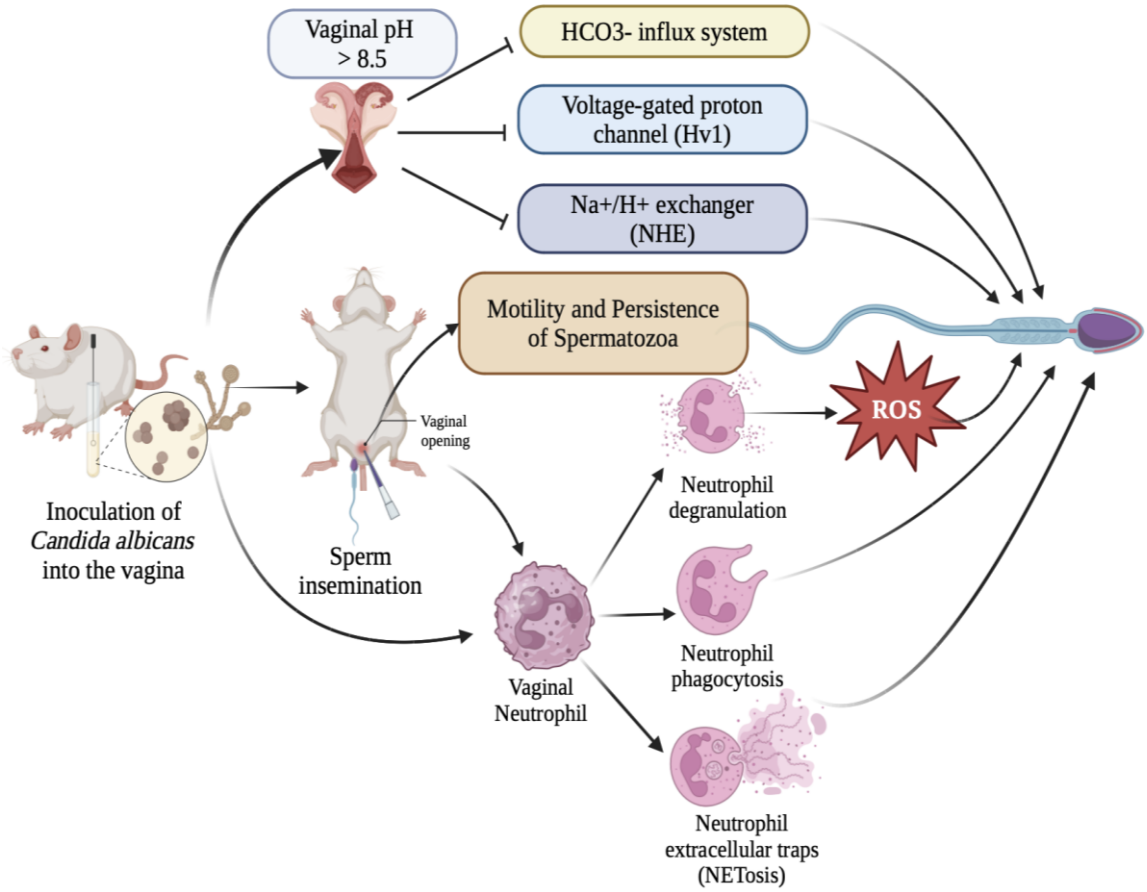

Figure 3. Proposed mechanism of the effect of pH and vaginal neutrophils on the motility and persistence of spermatozoa in the vagina of candidiasis rat models.

Conclusion

In summary, our study highlights that spermatozoa motility and persistence in candidiasis rat models are notably reduced compared to the normal group. We also found that elevated vaginal pH and increased neutrophil count in the candidiasis model contribute to this reduction in sperm motility and persistence. Additionally, vaginal pH and neutrophil count were both correlated with the motility and the persistence of spermatozoa.

Ethics approval

The research has received an ethical review and information about passing the ethical review from the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia/RSUPN dr. Cipto Mangunkusumo Number KET-1147/UN2.F1/ETIK/PPM.00.02/2023.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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