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# Medicolegal Aspects and Sperm Examination Procedures in Rape Cases: A Narrative Literature Review

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### 1. Introduction

Rape is an act of forced intercourse with a woman or man who is not their partner and is usually followed by violence and even murder. Rape is an event that is difficult to prove even though, in that case, a complete examination and collection of evidence has been carried out. Article 285 of the Criminal Code on rape states that: Anyone who uses violence or threats of violence forces a woman to have intercourse with him outside of marriage, is threatened with committing rape, with a maximum imprisonment of twelve years, so that in cases of rape, it must be proven first. There is intercourse. If intercourse cannot be proven, then it would be odd to say it was rape. Evidence in the form of sperm ejaculation

#### ABSTRACT

Sexual violence is usually a covert case where the witnesses are the victim and the perpetrator. For various reasons, even victims may not be able to provide complete information about the perpetrator or the identity of the rapist. With limited initial information, physical and biological evidence found from victims, crime scenes, and perpetrators will play an important role in the objective and scientific reconstruction of the incident. This literature review aimed to describe various sperm examination methods and medicolegal aspects of rape cases. Identification of one or more intact spermatozoa is conclusive evidence of the presence of semen, further confirming sexual contact. The conditions and the sperm collection area play an important role in determining the time interval between deposition and sample collection. Examination of spermatozoa can be done without staining and with staining. Examination of semen can be done by examination (tactile, visual, UV light, acid phosphatase reagent, zinc spot test, and baecchi stain).

obtained from rape victims will provide enormous assistance to law enforcement officials in the judicial process.<sup>4,5</sup> In the case of such evidence, forensic medicine can be used to reveal the perpetrators of sexual crimes.<sup>6</sup> This literature review aimed to describe various sperm examination methods in rape cases.

### Medicolegal aspects of proving rape cases

In an effort to prove by law that a crime of rape has occurred, in this case, forensic medicine plays a very important role in carrying out examinations and in obtaining medical explanations for events that occurred. Examination of rape cases in Indonesia is carried out by the Indonesian National Police (POLRI) as investigators to obtain evidence. Furthermore, the examination of the victim is submitted by a forensic doctor to examine the deceased rape victim, while the

survivors are examined by a specialist in obstetrics and gynecology.<sup>6,7</sup> The results of the examination are set forth in a visum et repertum which is useful for proving rape in court as documentary evidence or as expert testimony if the doctor is asked to appear in court.

Sexual violence is usually a covert case where the witnesses are the victim and the perpetrator.8 For various reasons, even victims may not be able to provide complete information about the perpetrator or the identity of the rapist. With limited initial information, physical and biological evidence recovered from the victim, from the crime scene, and from the perpetrator will play an important role in the objective and scientific reconstruction of the incident. The rampant cases of rape, even of minors, are often not accompanied by detailed information about the perpetrators and evidence of rape. 9,10 Indeed, it has become a culture in Indonesia rape is often considered a disgrace and embarrassment for the victim. This condition exacerbates the evidence of the case in the realm of jurisdiction as well as the psychological impact on the victim. 10

Liquid semen evidence is often a cornerstone of the investigation and prosecution of cases. 11 From various investigations of sexual violence, there are 4 main questions that can be assisted by a semen examination: Did sexual contact occur? When (or during what time) did sexual contact occur? Can specific actors be involved or not involved as potential sources of semen? Was the sexual contact forced or not? Therefore, the examination of semen can assist investigators in disclosing a rape case.

### Definition of spermatozoa and semen

Spermatozoa are mature cells of the male, specific products of the testes, which fertilize the mature ovum. 12,13 It is microscopic in size, looks like a translucent tadpole, has a flat elliptical head containing a rounded midsection, and a long tail that propels itself up with a powerful whipping motion. Identification of one or more intact spermatozoa is conclusive evidence of the presence of semen, further confirming sexual contact. The conditions and area of sperm collection play an important role in determining the time interval between deposition and collection (also known as the postcoital interval or PCI).

Semen is a complex mixture of the secretions of at least 4 male urogenital glands. <sup>13</sup> The seminal vesicle glands contribute approximately 60% to this mixture, the prostate gland approximately 30%, and the combined contribution of the epididymis and bulbourethral glands account for the remaining 10%. Only 10% of the volume of semen is sperm cells. Besides sperm cells, the rest of the semen is seminal fluid (90%), the extracellular fluid of the semen. Semen is the whole, thick, white liquid that comes out of the male genitals during ejaculation, called semen. <sup>14</sup>

# Sperm examination

### Sampling

Sperm examination begins with sampling. The liquid sperm sampling technique is as follows; the liquid is sucked in with a disposable pipette and then transferred to a sterile tube; or the liquid is absorbed with clean cotton, then dried in the air. Sampling in the form of spots on objects that can be moved, for example, pants, bed sheets, and pillows is as follows; if the spot is still wet, dry it in the air; if necessary, cut the part of the object with a clean knife or scissors; insert the cut in pocket paper. Meanwhile, if the spots are on objects that cannot be moved and the surface is not absorbent, then the spots are scraped off with a clean tool. Then the results of the scrapings are accommodated in clean paper and folded. Techniques for collecting sperm evidence on the bodies of victims of sexual crimes are as follows; victims are usually examined in hospitals; evidence can be found in the victim's mouth, vagina, and anus; Each item of evidence is placed in a separate container and labeled. All packaged samples were sent to the laboratory for analysis.15

### Spermatozoa examination method

Inspection methods were carried out with and without staining. Examination without staining is performed to see the motility of spermatozoa. This examination is most meaningful for estimating the time of intercourse. The inspection method is as follows; put one drop of vaginal fluid on the glass object, then close it. The sample is examined under a microscope with magnification 4 times and focused on the movement of spermatozoa. The results of the analysis are generally agreed that within 2-3 hours

after intercourse, spermatozoa can still be found moving in the vagina. Menstruation will extend this time to 3-4 hours. Other studies state that spermatozoa can still be found 3-6 days after intercourse. In dead people, spermatozoa can still be found up to 2 weeks after intercourse, maybe even longer.<sup>13</sup>

Sperm examination by staining is done to see sperm morphology. The inspection method is as follows; Make the smear and fix it by passing the glass smear over the flame. Samples were stained with hematoxylin-eosin, methylene blue or malachite green. An easy and good staining method for forensic purposes is staining with malachite green. The advantage of this stain is that the nuclei of the epithelial cells and leukocytes are undifferentiated, the epithelial cells are uniformly pink in color, and the leukocytes are not stained. The head of the spermatozoa appears red, the neck is pink, and the tail is green. If spermatozoa are not found, it is not certain that there is no ejaculation in the vagina because of the possibility of azoospermia or post-vasectomy. If this happens, it is necessary to determine the seminal fluid in the vaginal fluid.

### Malachite-green staining

Malachite green is an organic compound that is often used as a coloring agent. This substance is usually used by the textile industries and industrially traditionally used to dye materials such as silk fabrics, leather-based clothing, and paper. In Forensic science, malachite green is used in the leuco-malachite green (LMG) procedure, namely to detect the presence of latent blood. Aside from being a test reagent for the presence of latent blood, malachite green is also used to examine rape victims, which involves intercourse. The examination is intended to detect the presence or absence of sperm in cases of intercourse. 13,15

The inspection technique for painting malachite green is as follows; smear of vaginal fluid on a glass object, dry in air, fix with fire, stain with malachitegreen 1% in water, wait 10-15 minutes, wash with water, stain with eosin-yellowish 1% in water, wait 1 minute, wash with water, dry, examine under a microscope (Hoediyanto, 2012). The expected results on the Malachite–green staining are the purple sperm head base, the nose is pink, and on the Gram stain,

you will see sperm consisting of a reddish head, a bluish neck, and a tail. The result is declared positive if at least one intact sperm is found.

Sperm cells are rather difficult to distinguish from other complicating cells, which are also raised when the sample is taken. The complicating cells in question are vaginal wall epithelial cells and leukocytes. This resulted in the appearance under the microscopic between cells being rather difficult to distinguish, especially in thick smear preparations. Thick smears make it more difficult to distinguish sperm cell images because of the many types of cells that overlap.

Examination of sperm cells by staining requires 2 types of reagents, namely malachite green and eosin yellowish. The procedure tends to be longer. This examination cannot determine sperm motility due to fixation and staining processes. Nonetheless, the results are better. Staining makes it easier for the examiner to distinguish between target cells and troublesome cells. Malachite green does not color epithelial cells and leukocytes but is specific for the tail of spermatozoa.

Another dye used in preparations after malachite green is eosin yellowish. This reagent acts as a countering color which facilitates the examiner in getting rid of the complicating cells. Yellowish eosin gives a pink color to the epithelial cells. Leukocytes will not be stained. In addition, yellowish eosin will color the head and neck of the sperm cells.

The combination of malachite green stain and yellowish eosin counterstain gives a characteristic color to sperm cells. When it has been stained in such a way, spermatozoa will be easier to identify. The results of this typical smear make examination with staining superior in detecting sperm. <sup>16</sup>

Detection of spermatozoa with malachite green staining was considered very good because, based on the results of the study, within six days, spermatozoa could still be found intact and well stained. The same thing happens to sperm cells that are clustered and not intact. Although the organelles are not intact, the stain is still evenly distributed. Thus this study confirms the existing theory that malachite green is truly effective in detecting spermatozoa.

# Examination of the presence of seminal fluid (semen)

In certain situations, it can sometimes be difficult to find spermatozoa. For example, if the perpetrator suffers from azoospermia, which is a disorder in which there is no or almost no sperm in the semen, even though the perpetrator has repeated coitus, it will be difficult to find sperm. <sup>17</sup> In Conditions like this needs to use another method of examination, which is based on the composition of the semen, in the form of acid phosphatase from the prostate and choline crystals from the seminal vesicles.

### Acid phosphatase reaction

The acid phosphatase reaction is a screening test for the presence of seminal fluid, determining whether the spot is a seminal spot or not, so it must always be carried out on every sample suspected of being seminal fluid before other tests are carried out. An acid phosphatase reaction is carried out if the examination does not find spermatozoa cells. This test is not specific, and false positive results can occur in feces, tea, contraception, fruit juices, and herbs. The basic principle of the reaction is the presence of high levels of the enzyme acid phosphatase produced by the prostate gland. The enzyme acid phosphatase hydrolyzes sodium alpha naphthyl phosphate. The alpha naphthol that has been liberated will react with brentamine to produce azo dyes that are blue-purple in color. 14,15

Examination materials used in acid phosphatase reactions are vaginal fluids. The reagents used consisted of solution A and solution B. The composition of solution A was brentamine fast blue-B 1g, sodium acetate trihydrate 20 g, glacial acetic acid 10 ml, and distilled water 100 ml. The way to make it is as follows; The components of sodium acetate trihydrate and glacial acetic acid were dissolved in distilled water to produce a buffer solution with a pH of 5, then brentamine fast blue-B was dissolved in the buffer solution. The composition of solution B is 800 mg of sodium alpha naphthyl phosphate and 10 ml of distilled water. 89 ml of solution A plus 1 ml of solution B, then filter quickly into a dark bottle. If saved in the closet ice, the reagent can last for weeks, and any precipitate will not interfere with the reaction.

How to check seminal fluid using an acid phosphatase reaction, namely as follows; The suspected material is attached to filter paper which is first moistened with distilled water for a few minutes. Then the filter paper is removed and sprayed or dripped with reagent. The reaction time was determined from the time of spraying until the purple color appeared because the maximum color intensity was gradually reached. The test results on the spot that does not contain the phosphatase enzyme give a simultaneous color with a fixed intensity, while the spot containing the enzyme gives a gradual color intensity. A reaction time of 30 seconds is a strong indication of the presence of seminal fluid. If the reaction time is around 30-65 seconds, it still needs to be confirmed by electrophoresis. The reaction time of more than 65 seconds cannot be fully determined that there is no seminal fluid because a reaction time of more than 65 seconds has been found but positive spermatozoa. The enzyme acid phosphatase found in the vagina provides an average reaction time of 90-100 seconds. Pregnancy and the presence of bacteria or fungi can speed up the reaction time. 16,18

### Florence reaction

The Florence reaction is carried out when there is azoospermia or no spermatozoa are found, or other methods of determining semen cannot be done. The basis for the Florence reaction is the presence of choline in the seminal fluid, which is a product of the degradation of lecithin. With Lugol's solution, choline will react and form choline periodide crystals. The reagent (Lugol's solution) can be prepared from; potassium iodide 1.5 g, iodine 2.5 g, and 30 mL of distilled water. The inspection steps are as follows; spots extracted with a little distilled water. The extract is placed on the object glass, let dry, and covered with a cover glass. The reagent is dispensed with a pipette under the cover glass. The results are examined under a microscope. If semen is present, brown peryodida choline crystals appear in the form of needles with often split ends. This test is not unique to seminal fluid because materials of plant or animal origin will show similar crystals. However, a positive result on this test can determine the possibility that there is seminal fluid, and a negative result determines other possibilities besides seminal fluid.

### Berberio's reaction

This reaction is carried out and is meaningful if microscopic observations do not find spermatozoa. The basis of the reaction is to determine the presence of spermine in the semen. The reagent used is a saturated solution of picric acid. The examination method is the same as for Florence's reaction. The result is positive if you get yellowish picric spermine crystals in the form of a needle with a blunt tip. Sometimes there are lines of refraction that are located longitudinally. Crystals may also be ovoid in shape.

# Examination of seminal fluid with ultraviolet (UV), visual and tactile light

Examination of seminal fluid with UV is as follows; the material to be examined is placed in a dark room, then irradiated with UV light. If there is seminal fluid, it will appear white fluorescence. This method is not very effective because semen spots on nylon and silk materials do not fluoresce, and false positive results can also occur on detergent materials, vaginal secretions, and urine. 18

Examination of seminal fluid by the visual method is as follows; the object or material being examined is examined carefully whether there is a change in color or not. On non-absorbent textiles, if there is seminal fluid, it will appear shiny and translucent, whereas non-absorbent textiles will appear colorless or grayish.

Examination of seminal fluid by the tactile method is as follows; the object or material being examined and its entire surface is touched with the fingers or hand. On non-absorbent textiles, the surface will feel slightly stiff or rough if there are spots of semen (semen). Examination of semen by smell is positive if there is an odor resembling the smell of chlorine. <sup>16</sup>

## Spot zink test

The principle of the zinc spot test is the same as the acid phosphatase test. The presence of cement spots will give a pink result after spraying the spot zinc reagent. The components of the spot zinc reagent are as follows; 10 mg 1-(2-pyridylazo)-2-naphthol was dissolved in 2 ml Triton X-100 and mixed with 98 ml 0.5 M Tris (6 g Tris hydroxylmethyl) aminomethane solution in 100 ml distilled water. Storage is carried out in the refrigerator at 40°C.

### Baecchi's staining

In Baecchi staining, the reagent used consisted of 1% 1 ml fuchsin acid, 1% 1 ml methylene blue, and 1% 40 ml hydrochloric acid. The inspection steps are as follows; take clothes with spots in the middle (size 2x2cm); color with Baecchi for 2-3 minutes; wash with 1% HCl for 5 seconds; dehydrate with alcohol 70%, 85% & absolute, clear with Xylol and dry, place on filter paper; using a needle, 1-2 threads are taken for clothes that contain spots, then they are broken down and conveyed into fibers on a glass object; drop Canada balsam, cover with a lid, look under a microscope with 4x magnification. Baecchi staining results are positive if there are red and light blue spermatozoa heads and sperm heads attached to the thread fibers.

# Prostate-specific antigen (PSA) detection test in semen

Prostate-specific antigen (PSA) а glycoprotein produced by the prostate gland and secreted into the semen. PSA is one of the main proteins in semen, with concentrations between 0.2-0.3 mg/ml. The main function of PSA is to dilute the semen. This high level of PSA makes PSA a very useful biomarker in forensic medicine. The advantages of PSA examination in rape cases are as follows; PSA or p30 are acceptable markers for detecting semen in criminal cases; the PSA test is not a presumptive test like the phosphoric acid test on semen; PSA detection can be done even without finding the spermatozoa as in the case of men with azoospermia or who have had a vasectomy; a semen sample may show a positive PSA result even though the dilution factor is 1:200,000; PSA can be detected in urine after ejaculation and in the urine of adult men, and even in the urine of boys as young as 11 years old. 19,20

The sample used on the test PSA was in the form of a vaginal swab. The technique used is a quantitative test or rapid PSA testing. Quantitative tests are used to check sperm exposure in the last 48 hours. Rapid PSA testing is carried out using immunochromatography test strips. The result is positive when there is a pink line in both the test and control areas; negative if there is a pink line in the control area only.

### Semenogelin test

Semenogelin is a protein involved in the formation of matrix gel that coats the ejaculated spermatozoa. 20 There are 2 types of protein cementogelin, namely cementogelin 1 and cementogelin 2. The diagnosis process is almost the same as the PSA test, using rapid stain identification of human semen (RSID). The principle of this test is to use immunochromatographic that test uses two antibodies monoclonal specific to human cementogelin. In the cementogelin test, the semen must be evaluated exactly 10 minutes after the addition of the sample. The test result is declared positive if there is a red line respectively in the test and control areas.

### Legal aspects of sexual crimes

Crime against morality is every act done by someone which gives rise to sexual satisfaction, and on the other hand, these actions interfere with the honor of others.<sup>21</sup> Sexual crimes are crimes that arise and are obtained through intercourse. Intercourse is the insertion of the penis into the vagina, partially or completely, with or without ejaculation, at least through the vestibule. Fornication is any sexual assault without intercourse.

## The medicolegal procedure of investigation

Investigations are carried out by investigators, namely officials from the Republic of Indonesia National Police and certain civil servant officials who are given special authority by law-law as stipulated in Article 6 of the Criminal Procedure Code. 1-3 Investigators can ask for the help of an expert, and in the event of an incident involving the human body, the investigator can ask for the help of a doctor to carry out forensic medical treatment. The duties of a doctor include; (1) carrying out a forensic medical examination of the victim if officially requested by the investigator; (2) refusing to perform the forensic medical examination referred to above may be subject to imprisonment for a maximum of 9 months.3 The obligation to assist the judiciary as a forensic doctor is regulated in Article 133 of the Criminal Procedure Code, where, as stated above, investigators have the authority to submit requests for expert information from forensic doctors or judicial medicine. Meanwhile, sanctions for violators of doctor's obligations are regulated in articles 216, 222, 224, and 522 of the Criminal Code. Carrying out clinical, anatomical, and transplant post-mortem procedures by a forensic doctor is regulated according to government regulation No. 18 of 1981. A forensic doctor who makes a false statement in the final examination results is subject to Article 267 of the Criminal Code and Article 7.1-3

### 2. Conclusion

As stipulated in Article 6 of the Criminal Procedure Code, investigators can ask for the help of an expert, and in the event of an incident involving the human body, the investigator can ask for help from a doctor to carry out forensic medical treatment. The obligation to assist the judiciary as a forensic doctor is regulated in Article 133 of the Criminal Procedure Code. In an effort to prove by law that a crime of rape has occurred, in this case, Forensic Medicine plays a very important role in carrying out examinations and in obtaining medical explanations. An important role in the objective and scientific reconstruction of events, due to limited initial information, physical and biological evidence found from victims, from crime scenes, and from perpetrators. Spermatozoa and semen are often the cornerstones of investigations and prosecutions of cases. Examination of spermatozoa can be done without staining and with staining. Examination of semen can be done by examination (tactile, visual, UV light, acid phosphatase reagent, zinc spot test, and baecchi stain).

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