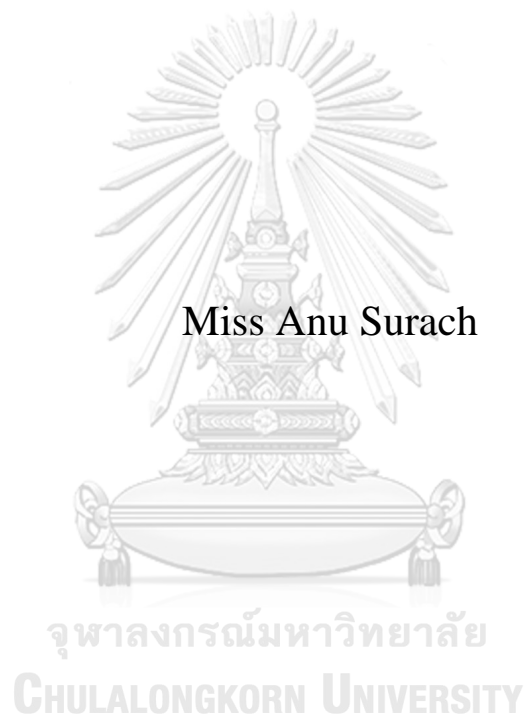Association between cotinine levels and the semen quality
among male tobacco farmers in Sukhothai Province, Thailand

Miss Anu Surach

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Public Health
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ความสัมพันธ์ของระดับโคตินินและคุณภาพอสุจิของเกษตรกรปลูกยาสูบชาย จังหวัดสุโขทัย ประเทศไทย

น.ส.อนุ สุราช

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาสาธารณสุขศาสตรดุษฎีบัณฑิต
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By   Miss Anu Surach
Field of Study   Public Health
Thesis Advisor   Associate Professor WATTASIT SIRIWONG, Ph.D.
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อนุ สุราช : ความสัมพันธ์ของระดับโคตินินและคุณภาพอสุจิของเกษตรกรปลูกยาสูบชาย จังหวัดสุโขทัย ประเทศ
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ยาสูบไทยเป็นปัญหาสาธารณสุขที่ใหญ่ที่สุดปัญหาหนึ่ง ใบยาสูบมีสารนิโคตินที่อาจส่งผลต่อคุณภาพชีวิตของ
เกษตรกรผู้ปลูกยาสูบ การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาหาความสัมพันธ์ระหว่างปัจจัยเกี่ยวข้องกับการทำงานกับระดับ
โคตินินในน้ำลายและคุณภาพอสุจิของเกษตรกรปลูกยาสูบ ในจังหวัดสุโขทัย ประเทศไทย เป็นการศึกษาแบบระยะยาว กลุ่ม
ตัวอย่าง คือ กลุ่มเกษตรกรชายปลูกยาสูบ จำนวน 62 คน ที่มีอายุระหว่าง 20-40 ปี ที่ไม่มีโรคทางระบบสืบพันธุ์ โรคเรื้อรัง
โรคอ้วน และโรคทางจิตเวช โดยทำการเก็บข้อมูล จำนวน 3 ครั้ง ในช่วงของการเก็บเกี่ยวใบยาสูบ ระหว่างเดือน มีนาคม ถึง
เดือน พฤษภาคม พ.ศ. 2565 เครื่องมือที่ใช้ในการทำวิจัยในครั้งนี้ ได้แก่ แบบสอบถาม เก็บตัวอย่างอสุจิ และเก็บตัวอย่าง
น้ำลาย สถิติที่ใช้ในการวิเคราะห์ข้อมูล ประกอบด้วยการทดสอบของฟรีดแมน, การวิเคราะห์ความแปรปรวนเมื่อมีการวัดซ้ำ
และการวิเคราะห์การถดถอยโลจิสติกแบบไบนารี่ ผลการศึกษาพบว่า ปริมาณน้ำอสุจิ, ความเป็นกรด-เบสน้ำอสุจิ, การเคลื่อนที่
ของตัวอสุจิ, รูปร่างของอสุจิ และจำนวนตัวอสุจิ ของเกษตรกรชายปลูกยาสูบในช่วงฤดูเก็บเกี่ยวใบยอดยาสูบและบ่มแห้ง ลดลง
เมื่อเทียบกับในช่วงฤดูเก็บเกี่ยวใบแรกยาสูบ และช่วงฤดูสิ้นสุดการเก็บเกี่ยว (p-value <0.05) ในขณะที่ระดับโคตินินใน
น้ำลายของเกษตรกรชายปลูกยาสูบในช่วงฤดูเก็บเกี่ยวใบยอดยาสูบและบ่มแห้ง สูงขึ้นเมื่อเทียบกับระดับโคตินินในน้ำลายของ
เกษตรกรชายปลูกยาสูบในช่วงฤดูเก็บเกี่ยวใบแรกยาสูบ และช่วงฤดูสิ้นสุดการเก็บเกี่ยว (p-value <0.05) เมื่อวิเคราะห์หา
ความสัมพันธ์ พบว่า อายุและการดื่มแอลกอฮอล์มีความสัมพันธ์กับระดับโคตินินในล้ำลาย ประสบการณ์ทำงานมีความสัมพันธ์
กับการเคลื่อนที่ของตัวอสุจิ ในขณะที่จำนวนชั่วโมงในการทำงานและการสวมถุงมือยางป้องกันสารเคมีมีความสัมพันธ์กับ
จำนวนตัวอสุจิ ข้อเสนอแนะในการศึกษา คือ ควรจัดโปรแกรมทางด้านสุขภาพเกี่ยวกับเรื่องความเสี่ยงต่อสุขภาพเพื่อลดการ
สัมผัสของเกษตรกรปลูกยาสูบ

สาขาวิชา	สาธารณสุขศาสตร์	ลายมือชื่อนิสิต ....
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		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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Anu Surach : Association between cotinine levels and the semen quality among male tobacco farmers in Sukhothai Province, Thailand. Advisor: Assoc. Prof. WATTASIT SIRIWONG, Ph.D. Co-advisor: Prof. Mark G. Robson, Ph.D.

Thai traditional tobacco is one of the greatest public health problems. Tobacco leaves contains nicotine that may impacts on the quality of life of tobacco farmers. The study aimed to explore the association of work-related factors on salivary cotinine levels and semen quality among male tobacco farmers in Sukhothai Province, Thailand. This was a longitudinal study of 62 participants aged 20-40 years without reproductive disease, chronic disease, obesity, and psychiatric disease in Sukhothai Province, Thailand. Data were collected three times during processing of cultivation tobacco growing in March to May 2022. A self-administered questionnaire was used to collect on individual characteristics, work related factors and personal protective behaviors. Semen samples were used to analyze for volume, pH, viscosity, motility, morphology, and sperm count and salivary samples were used to analyze for nicotine exposure levels. Data were analyzed using the Friedman Test, Repeated-measure ANOVA and Binary logistic regression. The outcomes revealed that semen volume, pH, motility, morphology and sperm count had a significant decreased from the picking top of tobacco plants and dry curing of tobacco plants (T2) than in the picking first of tobacco plants (T1) and the end of cultivation season period while the salivary cotinine levels of male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly higher than those of male tobacco farmers in the picking first of tobacco plants (T1) and male tobacco farmers in the end of cultivation season period (T3). Age and alcohol intake were significantly associated with salivary cotinine levels. Working experience was significantly positively associated with semen motility. Hour spent working and wearing gloves was significantly positively associated with sperm count. The study suggests that the need for public health intervention with health risk exposure to reduce farmer tobacco exposures from agricultural.

Field of Study:	Public Health	Student's Signature	....
Academic Year:	2022	Advisor's Signature	....
		Co-advisor's Signature	....

ACKNOWLEDGEMENTS

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CHAPTER I
INTRODUCTION

1.1 Background and Rationale

Tobacco is a leave of a grown plant that contains nicotine. There have been studies that measured the concentration of nicotine in the air in a variety of working environments for tobacco farmers. The results showed that nicotine concentrations in tobacco workplaces was very high. (Yoo et al., 2014) Nicotine is a chemical substance in the alkaloid group found in the leaves and stems of tobacco plants. Moderate smokers have a concentration of the daily nicotine level between 20-40 ng/mL the half-life of nicotine is average about 2-3 hours with nicotine being converted in the liver and is still slightly changed in the lungs and the brain.

Nicotine is transformed into cotinine. Cotinine is metabolized of by liver enzymes at the rate of around 70-80%, the remaining is metabolized in the lungs to become cotinine and nicotine N-oxide. Cotinine is a substance that has a very long half-life than nicotine, half-life of cotinine is 16-18 hours and having concentration levels in the bloodstream very high 10-15 times compared with nicotine. Therefore, in research studies it is commonly used to measure the amount of cotinine in the blood or saliva or urine to confirm nicotine in the body. (ChanthanaRangsingh, 2011)

The tobacco poisoning is one of the greatest public health disasters that kill more than 8 million people a year and over 7 million people die as a direct result of tobacco effects. (Organization & Control, 2008) The report of World Health Organization (WHO), regarding tobacco related deaths, indicates that we should be expecting more than 8 million cases per year in 2030. It is estimated that 10% of annual deaths worldwide is related to the use of tobacco products. (Organization & Control, 2008) Nicotine effects many systems of human body such as cardiovascular system, reproductive system, respiratory system, kidney, and others. (Mishra et al., 2015)

Besides, the study about tobacco effects on people showed that nicotine can affect the sperm characteristics (Condorelli et al., 2018); it reduces semen quality including volume, sperm motility, sperm morphology and concentration. (Asare-Anane et al., 2016) Even a low-dose of nicotine exposure at 0.6 mg/kg caused detrimental effects

on sperm characteristics. (Budin et al., 2017) The most important tool to diagnose and assess the semen quality is semen analysis including semen volume, sperm concentration, sperm count, sperm motility, and sperm motion. (Hwang, Walters, & Lipshultz, 2011)

Most of previous studies have been reported the relation between semen quality and smoking. It showed that the semen quality is affected in men who smoke. Another study has been reported that maternal smoking during pregnancy is related to decreased sperm concentration in children. In addition, the boys who have smoking fathers have high risk of lower sperm volume and the sperm count than the boys of a non-smoker fathers. (Axelsson et al., 2018)

The process of Thai traditional tobacco production involves seeding, transplanting to the tobacco filed, maintaining tobacco plants with fertilizer and pesticides applications, removing axillaries buds, cutting the top of tobacco plants, and removing weeds from the field. Tobacco leaves can be picked three times after planting. First time, it can be picked around 70-75 days after planting, the second time it can be picked around 80-85 days after planting, and the third time it can be picked around 90 days after planting. The tobacco farmers go to the field every morning to pick tobacco leaves and keep it into the place to incubate until they become dry. (Figure 1. show Tobacco Leaves Incubator) Tobacco leaves will become dry leaves around 2 to 4 weeks after picking.

Figure 1. Tobacco Leaves Incubator

According to the processing of Thai traditional tobacco cultivation, the farmers are exposed to the nicotine in tobacco leaves and might be at risks to its health effects.

Thailand is a developing country. The majority of population in the country is engaged in agriculture as part of the Thai way of life. In the old days, tobacco was intended for household consumption. Later, when Thailand developed the National Economic and Social Development Plan No. 1, it caused the change from cultivation for consumption in the household to commercial and industrial purpose, which included farming, gardening, as a main occupation. In addition, farmers have additional careers to provide income for the family and can be done throughout the year to circulate with crops that are the main occupation. Growing tobacco is another profession that farmers tend to do after the harvest during the month. October - April in Thailand.

According to the most areas in the north part of Thailand, such as Nan, Chiangmai, Chiang Rai, Payao, and Sukhothai are growing tobacco areas. The northeast part, such as Roi Et, Loei, Nong Khai and Chaiyaphum are also included. The top 3 most tobacco growing area are Phetchabun, Sukhothai and Roi Et (43,798 rai, 24,296 rai and 16,323 rai, respectively). They are also top producers quantitatively (9,279,300 kilograms, 6,398,017 kilograms and 3,414,470 kilograms, respectively) (Information and Communication Technology Center, 2019).

Traditional tobacco cultivation in Sukhothai is an air curing type called Burley tobacco. The tobacco leaves have different properties than other types. It has a transparent structure, absorbs aromatic, flavored water well. It has high nicotine content (2.5-4.0%). In this study we will assess the semen quality of nicotine exposure among male tobacco farmers. Cotinine levels exposure can be measured by evaluating the levels of metabolic metabolites in saliva.

The process of Thai traditional tobacco production involves seeding and planting in October to November, transplanting to the tobacco filed in November to December, maintaining tobacco plants with fertilizer and pesticides applications in November to February, harvesting tobacco leaves or picking tobacco leaves which can be picked three times after planting in March to April, and curing tobacco leaves in March to May.

The purpose of the study is to evaluate the salivary cotinine levels and semen quality different between the season periods among male tobacco farmers. Therefore, the researcher will collect the samples three times during processing of cultivation tobacco growing, it will be collected at first time in the harvesting tobacco leaves or picking tobacco leaves, to collect the second time in the harvesting process and drying process, and to collect the third time in end of tobacco cultivation.

According to the processing of Thai traditional tobacco cultivation, the farmers are exposed to the nicotine in tobacco leaves and might be at risks to its health effects. This investigation is a longitudinal study focusing on tobacco farmers who do work with processing of cultivation tobacco to describe the nicotine exposure, and consequences of salivary cotinine levels and measured semen quality change throughout the season periods year in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province. They represent the highest growing tobacco areas and tobacco growing households in Sukhothai Province.

It will describe the nicotine exposure, and consequences of salivary cotinine levels and measured semen quality change throughout the season. The data of this study will be helpful to extend surveillance of semen quality affect and improving working conditions of the farmers in the field.

1.2 Research Questions

1.2.1 Are there association between work-related factors and salivary cotinine levels among male tobacco farmers in Sukhothai Province, Thailand?

1.2.2 Is there association between salivary cotinine levels and semen quality among male tobacco farmers in Sukhothai Province, Thailand?

1.2.3 Are the salivary cotinine levels different between season periods among male tobacco farmers in Sukhothai Province, Thailand?

1.2.4 Are the semen quality different between season periods among male tobacco farmers in Sukhothai Province, Thailand?

1.3 Research Objectives

  1.3.1 General Objective

    1.3.1.1 To examine the association of work-related factors on salivary cotinine levels and semen quality among male tobacco farmers in Sukhothai Province, Thailand.

  1.3.2 Specific Objectives

    1.3.2.1. To explore the association between individual factors, work-related factors with salivary cotinine levels among male tobacco farmers in Sukhothai Province, Thailand.

    1.3.2.2 To explore the association between salivary cotinine levels with semen quality among male tobacco farmers in Sukhothai Province, Thailand.

    1.3.2.3 To evaluate salivary cotinine levels among male tobacco farmers in Sukhothai Province, Thailand.

    1.3.2.4 To evaluate the semen quality among male tobacco farmers in Sukhothai Province, Thailand.

    1.3.2.5 To evaluate the salivary cotinine levels different between the season periods among male tobacco farmers in Sukhothai Province, Thailand.

    1.3.2.6 To evaluate the semen quality different between the season periods among male tobacco farmers in Sukhothai Province, Thailand.

1.4 Research Hypothesis

<u>Null Hypothesis</u>

  1.4.1 There is no association between work-related factors and salivary cotinine levels among male tobacco farmers in Sukhothai Province, Thailand.

  1.4.2 There is no association between salivary cotinine levels and semen quality among male tobacco farmers in Sukhothai Province, Thailand.

  1.4.3 There is no difference of salivary cotinine levels between season periods among male tobacco farmers in Sukhothai Province, Thailand?

  1.4.4 There is no difference of semen quality between season periods among male tobacco farmers in Sukhothai Province, Thailand.

<u>Alternative hypothesis</u>

1.4.5 There is association between work-related factors and salivary cotinine levels among male tobacco farmers in Sukhothai Province, Thailand.

1.4.6 There is association between salivary cotinine levels and semen quality among male tobacco farmers in Sukhothai Province, Thailand.

1.4.7 There is difference of salivary cotinine levels between season periods among male tobacco farmers in Sukhothai Province, Thailand?

1.4.8 There is difference of semen quality between season periods among male tobacco farmers in Sukhothai Province, Thailand.

1.5 Scope of the Study

This study is a longitudinal study. Semen samples and saliva samples were collected three times during processing of cultivation tobacco. The study aims to investigate the factors association with semen quality among male tobacco farmers in Sukhothai Province, Thailand. The target population is male tobacco farmers aged between 20-40 years who do tobacco farm more than 1 years in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province.

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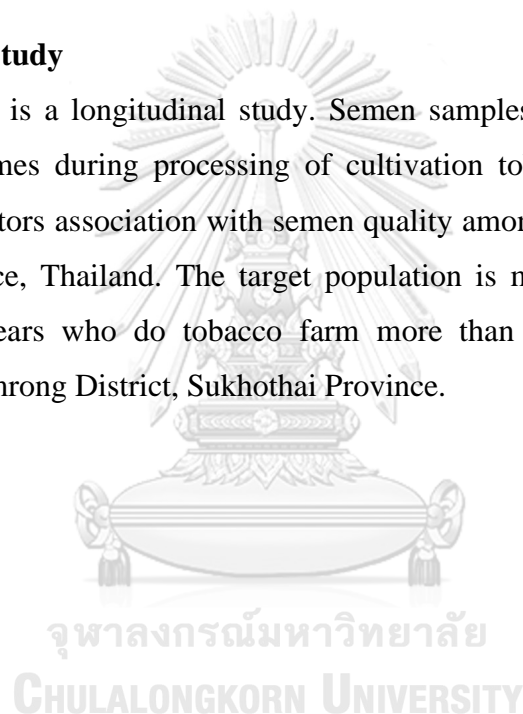
1.6 Conceptual Framework
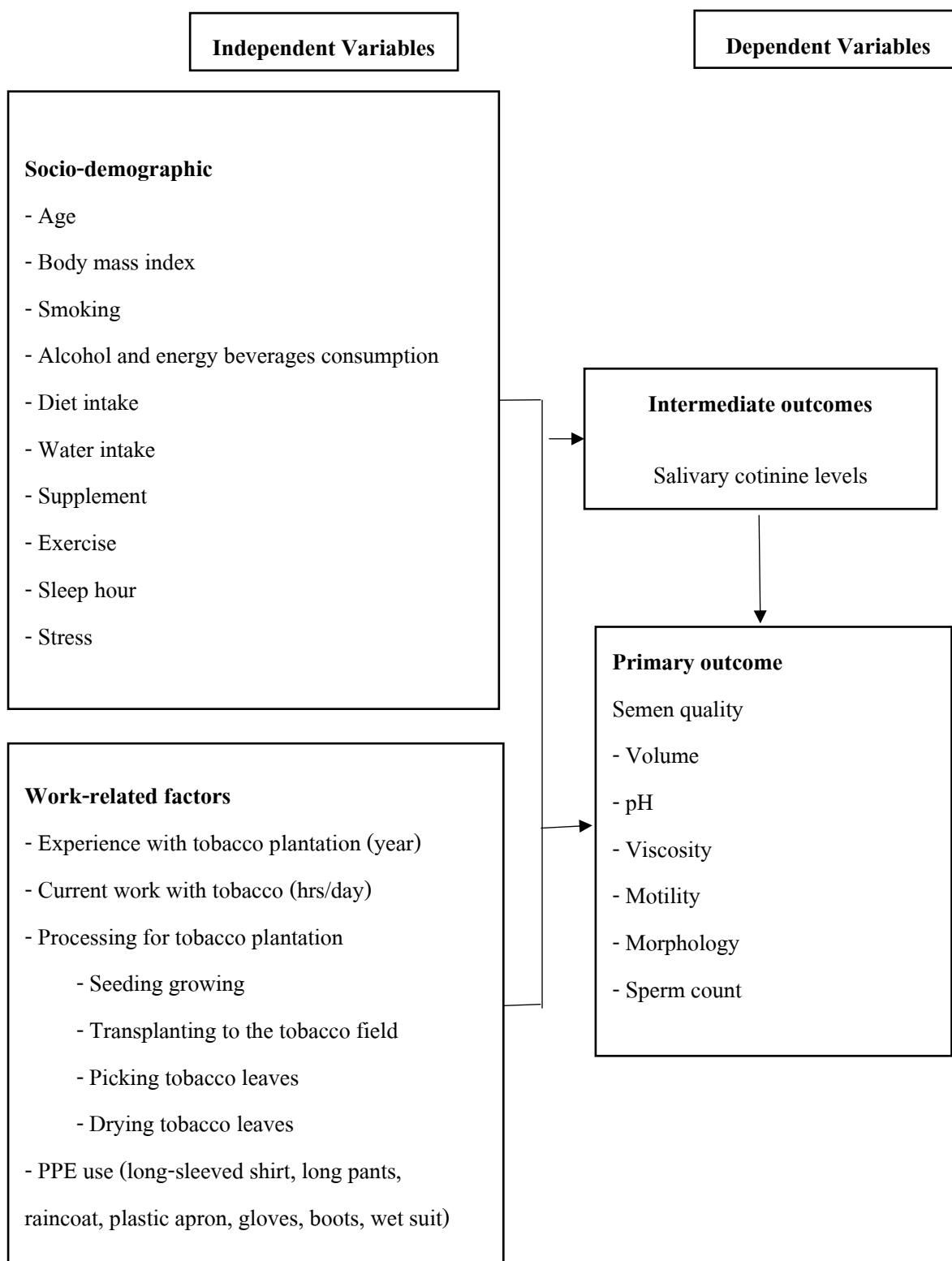
Figure 2. Conceptual framework

1.7 Operational Definitions

      1.7.1 Tobacco farmers are male tobacco farmers who live in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province.

      1.7.2 Work-related factors are the most important in association with work ability. In this study included are:

    - Seeding growing

    - Transplanting to the tobacco field

    - Picking tobacco leaves

    - Drying tobacco leaves

      1.7.3 Salivary cotinine levels are using by NicAlert, based on the principle of the enzyme that links the immunosorbent assay (ELISA). In this test, gold particles coated with monoclonal antibodies will be used with a set of traps. The thickness allows quantifying. The distance that the gold moves on the band will be known by clear color changes and provides accurate measurement of cotinine determination in saliva samples.

      1.7.4 Semen quality is a measure of male fertility which is a measure of the ability of sperm in semen for fertilization to be successful. Semen quality is related to both the quantity and quality of sperm, measured by using semen (volume, pH, viscosity, motility, morphology, and sperm count).

    - Volume. Normal ejaculation volume after 2-7 days of sexual omission is in the range from 2 to 6 ml.

    - pH. An alkaline coagulation liquid is the main component of semen. It releases from the seminal vesicles and flows through the ejaculate tubes. The normal range of Semen pH is 7.2 to 8.2. It will increase depending on the time after ejaculation.

    - Viscosity. Viscosity will be measured by the resistance of the flowing semen. High viscosity may hinder sperm motility, intensity and sperm antibody coating. Normally, semen will coagulate when water ejects and usually becomes liquid within 15-20 minutes.

    - Motility. The effective sperm passing through the cervical mucus depends on rapid movement, that is, the sperm must develop progression at least 25 μm / s forward.

- Morphology. The staining of semen allows the quantitative evaluation of the morphological form of normal and abnormal sperm in the ejaculate. The WHO method classifies irregular shaped sperm based on abnormalities of the head, tail and middle pieces, depending on the presence of sperm that recovers from cervical mucus and all border patterns that are considered abnormal.

- Sperm count is reported as being concentrated. (Million sperm per milliliter) including the total number of sperms (The concentration of sperm $\times$ milliliter of semen) in the ejaculation. Azoospermia means the absence of sperm in the semen. Oligozoospermia (often referred to as oligospermia) means the plasma semen concentration is less than 15 million per milliliter.

CHAPTER II
LITERATURE REVIEW

The researcher reviewed literature, concept, theory and research document involved in this study. It consisted of 4 parts namely:

Part I : Cultivation of tobacco

Part II : Nicotine

Part III : Male reproductive system

Part IV : Related Research

2.1 Cultivation of tobacco

2.1.2 History of tobacco

Tobacco has a central origin in Americas. Although humans have known tobacco for about two thousand years but they don't smoke regularly until the Indians who were Native Americans using tobacco widely, after that have a common tobacco farming.

The cultivation of tobacco in other sources began in the Haiti Islands in 2074 by receiving seeds from Mexico. And extended to neighboring islands until the year 2123 began to be planted in Cuba and continued to Guyana and Brazil in the late 22nd century Buddhist spread to Europe, Asia and Africa, there is evidence that humans in Ancient times, known as the planting of tobacco to be alley and rolling cigarettes. It is also known that tobacco has good antiseptic properties. The first country that started to grow tobacco in Asia is the Philippines. From then, it spread to India, China and Indonesia respectively.

The cultivation and planting of tobacco at that time there are about 10 variants of Verginia, but there are only 4 varieties that can be cultivated at that time: Hickory, White Berley, Maryland, and Joiner. The breeds were first cultivated.

Tobacco is a general term for any product that is prepared from the cured leaves of tobacco plants. There are more than 70 species. It contains nicotine-stimulating alkaloid. Dry tobacco leaves are used for smoking that are used as

chewing tobacco, dip tobacco and snus tobacco. Traditional tobacco cultivation in Sukhothai is an air curing type called Burley tobacco. The tobacco leaves have special properties than other types. It has a transparent structure, absorbs aromatic, flavored water well and good combustion properties in the production of American flavored cigarettes. The dried leaves are colored with flesh or light brown to dark brown. It has high nicotine content (2.5-4.0%) and low sugar content (0-4%). Also, it has light weight, good packing quality.

Table 1 show traditional tobacco cultivation area and production in Sukhothai Province by Si Samrong District season, the total cultivation area of provincial was around 24,296 rais and all the production of tobacco plant was around 6,398,071 kilograms. The most top three of tobacco cultivation and production were Thap Phueng Subdistrict, approximately 1,900,025 kilograms; Wang Thong Subdistrict, around 1,448,000 kilograms; and Ko Ta Liang Subdistrict, around 1,099,150 Kilograms, respectively.

Table 1. Traditional tobacco cultivation area and production in Sukhothai Province by Si Samrong District season 2019-2020

Subdistrict	Households	Area for cultivation (Rais)	Production (Kg)	Production average (Kg/Rai)
Khlong Tan	18	79	4,800	400
Wang Luek	121	712	187,430	399
Ban Na	495	2,486	569,369	394
Wang Thong	721	5,108	1,448,000	400
Koh Ta Liang	618	4,198	1,099,150	399
Wat Koh	69	430	114,497	391
Thap Phueng	1,234	7,345	1,900,025	398
Ban San	2	16	4,000	400
Wang Yai	538	3,922	1,070,800	400
Sum	**3,816**	**24,296**	**6,398,071**	**399**

Source: Plant production report form, Sisamrong District Agricultural Extension Office, November 2019.

Table 1 show traditional tobacco cultivation households in Sukhothai Province by Thap Phueng Subdistrict season, the total cultivation households of Thap Phueng Subdistrict were around 548 households. The most top three of household tobacco cultivation were Moo 4, approximately 123 households; Moo 3, around 122 households; and Moo 7, around 90 households, respectively.

Table 2. Traditional tobacco cultivation households in Sukhothai Province by Thap Phueng Subdistrict season 2019-2020

Thap Phueng Subdistrict	Households
Moo 1	38
Moo 2	60
Moo 3	122
Moo 4	123
Moo 5	24
Moo 6	28
Moo 7	90
Moo 8	1
Moo 9	62
Sum	**548**

Source: Plant production report form, Sisamrong District Agricultural Extension Office, November 2019.

2.1.2 Tobacco varieties

There are 3 varieties of tobacco using in Thailand which are Virginia tobacco species are characterized by a mild flavor. Burley tobacco species have a pungent flavor. Turkish tobacco species have a medium flavor.

Virginia Tobacco will have a mild flavor, it is a curing agent for hot cough incubated with hung through which is passed through the metal tube at the bottom of the curing oven curing the drug leaves that were impaled into a hanging panel

arranged in a line curing oven height. Time to incubate the dried leaves for about 5-6 days, while in the first 12-14 hours.

Amount of starch contained in the drug leaves will drop quickly. And later will be reduced slowly transform into whole sugar because heat helps to accelerate the enzyme. The nitrogen content almost unchanged the leaves are collected but ripe from the tree. After curing, there will be ripe orange or lemon scents. Virginia leaves is a drug containing high sugar content, low to medium nitrogen compounds, and has a comparable nicotine middle.

Burley tobacco varieties have a pungent flavor. It will be cooked in the fields as leaves then put it in a panel or cut the whole tree and hang it in the incubator to dry it by itself. Normally, it doesn't use heat. From the time when there is a lot of moisture in the atmosphere the cured leaves are colored. Light brown to red sugar with a pungent odor similar to the smell. Incubation of cocoa takes about 8 weeks. Sugar substances are slowly formed because enzymes are not accelerated and therefore curing leaves. Incubation of burley leaves is an incubation of air, which is to allow the leaves to slowly dry inside the incubator with temperature natural but control the humidity inside the incubator by closing the vents the reaction that occurred change.

Chemical composition in pharmaceutical leaves which is divided into 3 phase;

1. Yellow phase is the first phase that requires the leaves to wilt. turn yellow. It will take around 4-5 days.
2. During the brown phase, the color of the drug will turn yellow. Sugar slowly the drug leaves gradually dry. Accompanying will take about 10-12 days.
3. The final phase is drying. This phase will leave only moisture on the petiole, therefore should open the vent. The air to the max will take about 18-20 days.

Turkish Tobacco, or Eastern Drug has a medium flavor, using sun to incubate.

The leaves that are as small as the palm of the hand will give you the smaller leaves, the better quality. When collecting the leaves, starting from the base of 3-4 leaves at a time with a long rope, keep the wind for 3-4 days, then put it in the sun.

2.1.3 Processing of cultivation tobacco

The cycle crop of tobacco between 90 and 105 days, the season include plant, grow and harvest. Tobacco can adapt to the growth of various soils while the most suitable for cultivation are sandy loam and clay.

Tobacco can grow in various climates. The appropriate temperature for tobacco growing is between 20 to 30 degrees Celsius and also rainfall.

The processing of cultivation tobacco which are;

Planting season; The appropriate months to plant tobacco are November and December for the tropical climates. It is good to take advantage of the rain. The appropriate planting period in mild climate is in the spring and summer. The tobacco seeds are very small. It should be germinated in the green house before transplant in the field.

Green house should be installed in clean, well-drained location and near water source. When transplant the young tobacco plant to greenhouse should be careful process. Seedlings to be planted must be 3 to 6 inches tall after planting for 40-50 days.

Harvesting; The harvesting time depend on the variety of tobacco. The important thing is protection them from losing moisture.

Curing;, The tobacco leaves must be cured immediately after harvesting. The leaves are transformed into raw materials for the industry. (TobaccoAuthorityofThailand, 2015)

2.2 Nicotine Chemical substance

2.2.1 Nicotine and Related Alkaloids in Tobacco Products

Nicotine is a chemical substance in the alkaloids group found in the leaves and stems of tobacco plants. In general 1 roll of cigarettes contains 10-15 milligrams of nicotine.

Nicotine is a weak base property with a pKa value around 8.0 and can be dissolved well in water and fat. After a cigarette burns, nicotine is absorbed into the body. Nicotine is the main compound that the characteristics found in tobacco. It looks like oil with no color. It can absorb and dissolve in water then through the skin of humans and animals. It can spread through the blood-brain barrier.

The absorption of nicotine into the body depends on the acidity (pH) of the smoke produced. Nicotine is absorbed in the stomach little because of acidity but is well absorbed in the intestine, which has a weak base. Increasing the pH of the solution causes an increase in the concentration of non-charged lipophilic nicotine. In this form can pass through all the biological membranes. (Mishra et al., 2015) When nicotine enters the bloodstream, regardless of absorption at any location, nicotine can spread into the body that various organs can be physically, such as the brain, digestive tract, heart and blood vessels. When people smoke cigarettes, the body will receive nicotine into the bloodstream about 1-3 milligrams and enter the brain in about 7 seconds.

After a person inhales nicotine from tobacco, it will be carried particles into the lungs and rapidly absorbed. And it enters through the circulation into the brain so quickly. (Mishra et al., 2015)

The nicotine concentration in the arteries is higher than those found in veins up to 2-6 times, in which smokers in moderate amounts have a concentration of the daily nicotine levels are between 20-40 ng/mL. After that, the concentration of nicotine in the bloodstream will decreased rapidly because it spread into peripheral tissues. The half-life of nicotine is average about 2-3 hours with nicotine being converted in a liver and is still slightly changed in the lungs and brain. Nicotine will be transformed into cotinine, which has a much less nicotine effect.

Cotinine is a substance that has a very long half-life than nicotine, half-life of cotinine is 16-18 hours and having concentration levels in the bloodstream very high 10-15 times compare with nicotine. Therefore, so in research studies about commonly used to measure the amount of cotinine in the blood or saliva or urine to confirm nicotine in the body. (ChanthanaRangsingh, 2011)

Cotinine is a metabolism of nicotine by liver enzymes, it is around 70-80%, the remaining is metabolized in the lungs to become cotinine and nicotine N-oxide. The human kidneys drive the cotinine and other metabolites from the body. Therefore, the detection of cotinine by using urine. Cotinine can be excreted in approximately 76% of the kidneys.

A low-dose nicotine exposure at 0.6 mg/kg caused detrimental effects on sperm characteristics. (Budin et al., 2017)

2.2.2 Mechanism of Nicotine

2.2.2.1 Pathways of Nicotine and Cotinine Metabolism

Nicotine is extensively metabolized to a number of metabolites by the liver. Six primary metabolites of nicotine have been identified. Quantitatively, the most important metabolite of nicotine in most mammalian species is the lactam derivative, cotinine. In humans, about 70– 80% of nicotine is converted to cotinine. This transformation involves two steps. The first is mediated primarily by CYP2A6 to produce nicotine-Δ 1′ (5′) -iminium ion, which is in equilibrium with 5′-hydroxynicotine. The second step is catalyzed by a cytoplasmic aldehyde oxidase. Nicotine iminium ion has received considerable interest since it is an alkylating agent and, as such, could play a role in the pharmacology of nicotine (Shigenaga et al. 1988).

Although on average about 70–80% of nicotine is metabolized via the cotinine pathway in humans, only 10–15% of nicotine absorbed by smokers appears in the urine as unchanged cotinine (Benowitz et al. 1994). Based on studies with simultaneous infusion of labeled nicotine and cotinine, it has been determined that 70–80% of nicotine is converted to cotinine (Benowitz and Jacob 1994). About 4–7% of nicotine is excreted as nicotine N′–oxide and 3–5% as nicotine glucuronide (Benowitz et al. 1994;Byrd et al. 1992). Cotinine is excreted unchanged in urine to a small degree (10–15% of the nicotine and metabolites in urine). The remainder is converted to metabolites, primarily trans–3′– hydroxycotinine (33–40%), cotinine glucuronide (12–17%), and trans–3′–hydroxycotinine glucuronide (7–9%).

The rate of metabolism of nicotine can be determined by measuring blood levels after administration of a known dose of nicotine (Hukkanen et al. 2005c). Total clearance of nicotine averages about 1200 ml per min. Nonrenal clearance represents about 70% of liver blood flow. Assuming most nicotine is metabolized by the liver, this means that about 70% of the drug is extracted from blood in each pass through the liver. The metabolism of cotinine is much slower than that of nicotine. Cotinine clearance averages about 45 ml/ min.

2.2.2.2 Route of Nicotine Absorption

Oral; The bioavailability of nicotine in humans following oral intake ranged from 24 to 59%. Following absorption, peak concentrations of nicotine in human

plasma were found at 3-6 hours after dermal application and at 90 minutes after oral administration.

Inhalation; The percentage of uptake of nicotine the compound through the lungs is 60 to 80%.

Dermal; The average dermal absorption of nicotine, measured in human volunteers who were treated with nicotine patches, was 18% over 24 hours.

2.2.3 Nicotine Toxicity

2.2.3.1 Acute toxicity; In experimental animals, the dose of nicotine which is lethal to 50% of the animals (LD50) varies widely, depending on the route of administration and the species used. The intravenous LD50 dose of nicotine in mice is 7.1 mg per kg. By direct intravenous administration the LD50 to rats was determined to 1 mg per kg. The intra peritoneal LD50 values for nicotine in mice and rats have been found to be 5.9 mg per kg and 14.6 mg per kg, respectively. The oral LD50 dose for nicotine in rats is 50 mg per kg to 60 mg per kg. The wide variation in sensitivity to the toxic effect of nicotine in rodents appears to be genetically determined. Dermal acute toxicity (LD50) in rabbit is 140 mg per kg. In interpreting animal toxicity data it is important to recognize that the route of administration is an important determinant of toxicity. Rapid intravenous injections result in the highest blood and brain concentrations and produce toxicity at the lowest dose. In contrast, oral or intraperitoneal administration requires higher dose to produce toxicity. This is due in part to pre-systemic metabolism of nicotine whereby, after absorption into the portal venous circulation. Probable oral lethal dose in human is less than 5 mg per kg or a taste (less than 7 drops) for a 70 kg person. It may be assumed that ingestion of 40 mg to 60 mg of nicotine is lethal to humans. (US-EPA. 1987). No inhalation toxicity data are available on which to base an immediately dangerous to life or health concentration (IDLH) for nicotine. Therefor, the revised IDLH for nicotine is 5 mg per m^3 base on acute oral activity data in humans and animals. (Trimble, 1994)

2.3.1.2 Long-term toxicity;

Reproductive toxicity

Cigarette smoking has major effects on the reproductive potential of humans. It has an anti-oestrogenic effect in women. (Tankó & Christiansen, 2004) Women

who smoke have significantly more variable segment and menses length than non-smokers, with heavy smokers ($20 cigarettes per day) running a risk of shorter segment length than non-smokers due almost entirely to the shortening of the follicular phase. (Windham et al., 1999) The likelihood of irregular cycles increases with the number of cigarettes smoked. (Kato et al., 1999) These effects decrease fertility in women as well as reduce the age of menopause. Menopausal symptoms such as hot flushes are experienced more commonly among smokers. (Staropoli, Flaws, Bush, & Moulton, 1998) Nicotine can inhibit hormones that cause hypothyroidism. It destroys the metabolic to androgens formation. This cause to irregular menstrual cycles. And there found the studied that cigarette smoking in women had estrogen and progesterone decreases. (Jin, Roomans, & pathology, 1997)

In males, the effect of smoking on androgen levels is important, given the recent interest in the association between low androgen levels and the metabolic syndrome, and coronary heart disease. (Pugh, Jones, West, Jones, & Channer, 2004) Various studies examining the effects of smoking on serum testosterone levels have reported conflicting findings largely due to difficulties in the hormonal assays. Nicotine can affect the Nitrous oxide synthesis to be impairment. And nitrous oxide derived from endothelial cells to helps in the formation of the male organ. It may lead to the loss of penile erection and erectile dysfunction. (Xie, Garban, Ng, Rajfer, & Gonzalez-Cadavid, 1997)

Genotoxicity and carcinogenicity

Nicotine was reported to increase chromosome aberrations and sister chromatid exchange frequency in a dose- and time-dependent manner in Chinese hamster ovary cells, and it was concluded that nicotine acted as a clastogen. It was also reported that nicotine was genotoxic at the concentrations found in saliva achieved during tobacco chewing. (Trivedi, Dave, & Adhvaryu, 1990) Nicotine was found as a co-carcinogen in animals. (Policy & Analysis, 1987)

Cardiovascular disease

Nicotine plays an important role in the development of cardiovascular disease. It could promote atherosclerotic disease by its actions on lipid metabolism and coagulation, by haemodynamic effects, and/or by causing endothelial injury. Compared to nonsmokers, cigarette smokers have elevated low-density (LDL) and

very-low-density lipoproteins (VLDL), as well as reduced high-density lipoprotein (HDL) levels, a profile associated with an increased risk of atherosclerosis. Nicotine increases heart rate through the activation of the sympathetic nervous system. (Health & Services, 2006) Lipid peroxidation and generation of free radicals, increased in smokers, are the processes associated with the pathogenesis of atherosclerosis. The products of lipid peroxidation may cause irreversible damage to the membrane structure of the cells. Some studies show that nicotine administration to animals results in endothelial cell abnormalities and decreases the synthesis of prostacyclin (an inhibitor of platelet aggregation). (Pittilo, 1990) Nicotine can increase heart rates, blood pressure and cardiac contractions. It can help to decrease blood flow in the coronary arteries and increase blood flow in skeletal muscles. (Kaijser & Berglund, 1985)

Pulmonary toxicity

Cigarette smoking is the major cause of chronic obstructive lung disease. (Health, Human Services %J Public Health Service, & No., 1984) Nicotine, which is readily absorbed from the lung and distributed to tissue, including bone marrow, increases the expression of the elastase gene, leading to increased elastase protein concentration per cell, suggesting a pathophysiologic mechanism for emphysema. (Armstrong et al., 1996) Inhaled nicotine produces a concentration dependent cough and airway obstruction in healthy subjects, probably because of stimulation of afferent nerve endings in the bronchial mucosa and mediated through parasympathetic cholinergic pathways. (Hansson, Choudry, Karlsson, & Fuller, 1994)

Gastrointestinal toxicity

Normally, the gastrointestinal mucosa is protected from injury by a layer of mucus and by the secretion of bicarbonate by gastric and duodenal epithelial cells to neutralize gastric acid. If these protective mechanisms are impaired, or if there is an increase in the levels of damaging factors, then ulceration may occur. Nicotine and other components of cigarette increase the reflux of duodenal contents into the stomach and mouth, decrease the secretion of pancreatic bicarbonate, decrease the production of gastric mucus and cytoprotective prostaglandins, and perhaps increase the production of free radicals and the release of vasopressin, a potent vasoconstrictor. (Eastwood, 1997) Nicotine can affect to Gastro Esophageal Reflux Disorder (GERD)

and peptic ulcer disease. (Chu, H Cho, & Y Shin, 2013) It decreases the tone of the large intestine and gastric movement and reduce the pressure of the lower esophageal sphincter, it may be the cause of the increased incidence of Gastro Esophageal Reflux Disorders. (Kadakia, De la Baume, Shaffer, & sciences, 1996) Nicotine can increase glycogen synthesis. It leads to reduce fasting blood glucose levels. And it affects to insulin resistance metabolic syndrome. (Somm et al., 2008)

Immunological system

The action of the hypothalamo-pituitary glands and stimulation of the autonomic nervous system through the paths of sympathy and parasympathetic affects the immune system which causes immune deficiency. (Sopori et al., 1998)

Nicotine is immune via the central and peripheral mechanisms. It can cause a decrease in the antigen and receptor mediator in the lymphatic system leading to decreased immune responses. (Geng et al., 1995)

2.2.4 Biomarkers of Nicotine Exposure

Nicotine measurement is highly specific for tobacco use or exposure (in the absence of nicotine medication use), but because of nicotine's short half-life (2 hours) the method is not recommended for general use. Cotinine is a highly specific and sensitive marker for tobacco use (in the absence nicotine medication use) and has the advantages of a fairly long half-life (16 hours).

A harmful contamination of the air can be reached rather quickly on evaporation of this substance at 20 °C resulting in nausea, vomiting, convulsions, abdominal pain, diarrhoea, headache, sweating, weakness, dizziness and confusion. Nicotine irritates the eyes and the skin and may cause effects on the cardiovascular system and central nervous system, resulting in convulsions and respiratory failure. Exposure far above the observed effect level may result in death. The effects of nicotine may be delayed, so medical monitoring is indicated. Acute toxic effects from nicotine generally result from oral exposure. (Brčić Karačonji, 2005) Nicotine is highly toxic with nausea occurring from exposure to 2 mg to 5 mg and deaths were reported in adults from ingested quantities of 40 mg to 60 mg. Infants are especially susceptible to nicotine toxicity. The ingestion of one or more fresh cigarettes is considered potentially toxic. Chronic toxicity that may be caused by prolonged exposure to small doses occurs in smoking. (Policy & Analysis, 1987) Workplace

level of nicotine in the air due to environmental tobacco smoke (ETS) is 20 µg per m^3. The dose of nicotine inhaled is equal to the product of air concentration and ventilation rate. A typical ventilation rate for an adult during light activity is 1 m^3 per hour. Thus, the intake of nicotine would be about 20 µg per hour. About 71 % of nicotine that is inhaled is absorbed, so the systemic dose of nicotine is estimated to be about 14 µg per hour. Assuming an eight-hour workplace exposure, this would be equivalent to 112 µg per day. (Coultas, Samet, McCarthy, & Spengler, 1990) The estimated absorption of nicotine from this level of exposure over nine hours is 55 µg. In office workplaces that banned smoking, the median air nicotine level was 0.3 µg m-3. The National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Limit (REL) and the American Conference of Governmental Industrial Hygienists' (ACGIH) Threshold Limit Value (TLV) for nicotine are 500 µg m-3. (Policy & Analysis, 1987) A model used to derive a health-based standard for environmental tobacco smoke (ETS) has shown that an eight-hour, time-weighted average exposure to 2.3 µg per m^3 of nicotine would correspond to three lung cancer deaths among 10,000 exposed people over a working lifetime. (Trout et al., 1998)

Average nicotine daily intake (i. e. absorbed dose) from significant environmental tobacco smoke (ETS) plus dietary exposure is about 80 µg and even a diet rich in nicotine-containing food is only 10 % of the total nicotine exposure. (N. L. J. E. h. p. Benowitz, 1999)

In 2004, The Committee on Updating of Occupational Exposure Limits (6) considered the noobserved-adverse-effect level (NOAEL) of 0.5 mg m-3 from a two-year inhalation rat study as a starting point in deriving a health-based recommended occupational exposure limit (HBROEL). The Committee noted that the actual NOAEL might be higher since exposure was for 20 h a day and only one concentration was tested. Since workers are supposed to be exposed for maximally eight hours a day, this NOAEL is adjusted, resulting in a 1.25 mg m-3. For the extrapolation to a HBROEL, the Committee established an overall assessment factor of 9. This factor covered intraand interspecies variation. Thus, applying this factor of 9 and the preferred-value approach, a healthbased occupational exposure limit of 0.1 mg m-3 was recommended for nicotine. The Committee recommended a health-based occupational exposure limit for nicotine of 0.1 mg m-3, as an eight-hour time-

weighted average (TWA). Because of the high skin absorption potential of nicotine, the committee advised a skin notation. After the final report was published in March 2004, the Health Council received comments which were taken into account in deciding on revised version published in 2005. The committee considered NOAEL of 0.5 mg m-3, implying that this two-year inhalation rat study does not provide information on the lowest exposure level at which adverse effects are becoming manifest. Therefore, the committee considered this study inappropriate for deriving a health-based occupational exposure limit (56). The current occupational exposure limit for nicotine in Croatia is 0.5 mg m-3. (Brčić Karačonji, 2005) The risk of occupational exposure is low if protective measures are applied (eye protection in combination with breathing protection, protective gloves and clothing, and ventilation).

2.2.4.1 Cotinine as a Biomarker for Intake of Nicotine

The presence of cotinine in biological fluids indicates exposure to nicotine. Because of the long half-life of cotinine it has been used as a biomarker for daily intake, both in cigarette smokers and in those exposed to secondhand tobacco smoke (Benowitz 1996). There is a high correlation among cotinine concentrations measured in plasma, saliva, and urine, and measurements in any one of these fluids can be used as a marker of nicotine intake. There is, however, individual variability in the quantitative relationship between steady state cotinine levels and intake of nicotine. This is because different people convert different percentages of nicotine to cotinine (usual range 50–90%), and because different people metabolize cotinine differently at different rates (usual clearance range 20–75 ml/min) (Benowitz 1996). While cotinine metabolism is affected by factors such as race, gender, age, genetic variation in the liver enzyme CYP2A6, and/or by the presence of pregnancy, liver or kidney disease. Another limitation to the use of cotinine is that, given an average half-life of 16 h, cotinine levels reflect relatively short-term exposure to tobacco (that is, over the past 3–4 days).

2.2.4.2 Nicotine and Cotinine in Hair and Nails

The use of hair as a material in which to measure nicotine and cotinine has been proposed as a way to assess long-term exposure to nicotine from tobacco products. Nicotine and cotinine are incorporated into hair as it grows over time. The

average rate of hair growth is 1 cm per month. Thus, measurements of levels of nicotine may provide a way of assessing exposure of a person to nicotine over several months (Al-Delaimy et al. 2002; Florescu et al. 2007).

Potentials problems with the use of hair include a strong influence of hair pigmentation on nicotine and cotinine binding and uptake (Dehn et al. 2001). Nicotine and cotinine are bound to melanin. As a result, dark hair binds much more nicotine than does blond or white hair. This makes comparison across individuals difficult. Also, hair is exposed to nicotine and cotinine from sweat and from sebaceous gland secretions, and to nicotine from environmental tobacco smoke exposure. Washing the hair before analysis may reduce this problem of environmental contamination, but it is not likely to remove all environmental nicotine and cotinine.

2.2.4.3 Dietary Sources

Dietary sources of nicotine have been alleged to be a potential confounder of cotinine levels used in measurement of secondhand smoke exposure. Several foods contain small amounts of nicotine (Siegmund et al. 1999). However, the levels of nicotine in foods are quite low. Based on nicotine levels in foods and the usual daily consumption of various nicotine-containing foods, it has been determined that the levels of cotinine produced by even a diet high in nicotine containing foods is lower than that seen in individuals exposed to moderate levels of secondhand smoke (Benowitz 1996).

2.2.4.4 Optimal Cotinine Cut-Points to Distinguish Tobacco Use from No Tobacco Use

Based on the work of Jarvis and coworkers, who measured cotinine levels in individuals attending outpatient clinics in the United Kingdom in the early 1980s, an optimal plasma or saliva cotinine cut-point of 15 ng/ml or a urine cotinine of 50 ng/ml were determined to discriminate smokers from nonsmokers (some of whom are exposed to secondhand smoke) (Benowitz et al. 2002a). Data from the National Health and Nutrition Examination Surveys (NHANES) from 1999 to 2004 were recently analyzed to assess the optimal serum cotinine in the US population at present (Benowitz et al. 2008a). Using receiver operator characteristic curve analysis, the optimal cotinine cut-points were 3.08 ng/ml for adults (sensitivity 96.3%, specificity 97.4%) and 2.99 ng/ml for adolescents (sensitivity 86.5%, specificity 93.1%).

2.2.5 Salivary Cotinine levels

The half-life of nicotine is average about 2-3 hours with nicotine being converted into a liver and is still slightly changed in the lungs and brain. Nicotine will be transformed into cotinine, which has a much less nicotine effect. Cotinine, the major proximate metabolite of nicotine, is n widely practiced as a biomarker of tobacco exposure. (N. L. J. E. r. Benowitz, 1996) Cotinine is a metabolism of nicotine by liver enzymes, it is around 70-80%, the remaining is metabolized in the lungs to become cotinine and nicotine N-oxide. The human kidneys drive the cotinine and other metabolites from the body. Cotinine is a substance that has a very long half-life than nicotine, half-life of cotinine is 16-18 hours and having concentration levels in the bloodstream very high 10-15 times compare with nicotine. Therefore, so in research studies about commonly used to measure the amount of cotinine in the blood or saliva or urine to confirm nicotine in the body. (ChanthanaRangsingh, 2011) Cotinine can be widely used in further compared to other diagnostic tools because of its higher sensitivity, specificity, long half-life as well as it is the best indicator for distinguishing the tobacco users from non-users. (Raja et al., 2016)

The small molecules, minimal protein binding in blood and water solubility increases concentration of cotinine in saliva by 15% to 40%. Thus cotinine measurement in saliva becomes a non-invasive, easy and well tolerated collection procedure when multiple samples are required over a limited period. (Avila-Tang et al., 2013)

2.3 Male reproductive system

2.3.1 Anatomy and physiology of male reproductive

The male reproductive system are the reproductive organs, external genital organs, reproductivity system and additional glands (Fig.2.3).
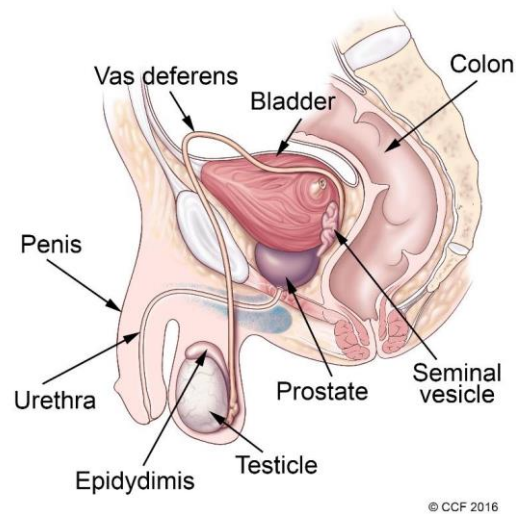
Figure 3. Parts of male reproductive system
Source:https://www.clevelandclinic.org/healthinfo/ShowImage.ashx?PIC=4106&amp

The male reproductive system has two major function. The first is spermatozoa, and the second is shipping of the male gametes into the female reproductive system (Table 3).

Table 3. Outlines the components of the male reproductive system

Components of the male reproductive system	
External genital organ	Penis, scrotum
Gonads	A pair of testicles
Reproductive tracts	Epididymis, vas deferens, ejaculatory duct, penile part of urethra
Accessory sex glands	Prostate, seminal vesicle, and bulbourethral glands

Penis is a sex organ which acts as a source of convulsions and rheumatism. The muscles are smooth and the capillaries are divided into 3 parts including root, body and gland penis. (Woodhouse CR, 1984)

Scrotum is having a bushy appearance on the lower, no fat in this area. The wall is smooth, wrinkled when contracted. There are pigments which are very pigmented. It's function to protect and control the temperature for the testes.

Testes is the main reproductive organs or genital organs of male. The shaped like an egg 2 months before giving birth, testicles descend into the testicle cyst (scrotum). The testicles are connective tissue which is the Tunica Albuginea into the testicle including Seminiferous tubules and Interstitial cells. They are sperm production (gametogenesis) and testosterone production (steroidogenesis). (Johnson, 2012)

Semi-ferrous pipes can be divided from the outside into 2 layers: the basement membrane is the base of the cell lining with a layer of lining the basement. The cells of the lining are cells of magnitude.

Larger than germ cells, called the Sertoli cell is believed to be the cells that produce nutrients to sperm. Sometimes this cell is called a sustained cell or nurse cells.

Each testicle has a seminiferous tube of approximately 400-600, 70-80 cm long and 0.12–0.3 mm in diameter. Each tube is surrounded by a base sheet which has a stratified epithelium consisting of stem cells, sperm cell formation and cells.

Epididymis is consist of a C-shaped tube coiled back and forth about 4-6 cm long in the testes. It is a temporary resting place for sperm to collect until it grows.

Spermatic cord is a collection of vessels, nerves, remnants of vaginal processes, and vas deferens, which suppress the testicles in the scrotum. The left cord is slightly longer than the right cord that causes the left testicle to be lower than the right side. (Moore, Dalley, & Agur, 2013)

Vas Deferens are connected with epididymis into the abdominal cavity. There are 2 ducts behind the stomach each pipe is 45 cm long. Before the end of the pipe, there is a bulging design called Ampulla to connect with the seminal vescicle and the ejaculatory duct. Vasectomy will
bind 2 tubes of Vas deferens to prevent the secretion of sperm to the outside.

Seminal vesicles are a pair of glands accessories that are placed between the bladder and anus. The length is about 5 cm. It has a function to secret liquid that is

base to control the pH of Semen and make food for sperm which is sugar Fructose and Vitamin C. (Standring, 2015)

Male urethra can be divided into three parts: the urethra, the membrane of the urethra and the male urethra. The length is approximately 20 cm and protruding from the neck of the bladder to the outer flesh of the glans penis.

Prostate Gland is in the area around the upper part of the gastric tube that starts out of the bladder and in front of the anus duct. There is 5 lobe and the weight is around 20 grams. It creates a white liquid like milk which is a mild base, helping to reduce sperm to be suitable for conditions.

The ejaculation tube is approximately 2 cm long which occurs on both sides by the tube of the seminal vesicle.

Ejaculation is a process, in which the spermatozoa mixed with seminal fluid is excreted from the urethra to the penis, while sexual excitement, the external urethra of the penis is moist due to secretion of the Bulbourethral gland.

At the time of orgasm, friction in the glans penis and the stimulation of sympathetic nerve fibers, delivering the smooth muscles of the seminal tubes, vas deferens, seminal vesicles and prostate glands. According to that, the contract for smooth muscles and sperm along with ejaculation from the seminal vesicles and the prostate are released into the urethra.

Rhythmic contraction of muscles bulbospongiosus compression of the urethra, according to the result which the semen is expelled into antegrade segment from the penile urethra. While the process, the reverse flow of semen into the bladder is protected by contractions of the bladder sphincter. (Giuliano & Clément, 2005)

2.3.2 Physiology of spermatogenesis

The male reproductive system has the function under the control of the gonadotropin from the anterior pituitary gland, which includes the intercellular cells, stimulating hormones, or ICSH, also known as Lutinizing hormone (LH) follicle stimulating hormone (FSH), androgen (or testosterone), prolactin and other related hormones Usually FSH encourages sperm production. CSH stimulate Growth of Leydick cells and stimulate the production of androgen hormones, the male hormones, which are hormones that are made from the testes including reproduction system and sperm production.

The function of hormones from the pituitary gland that controls the function of testicular cells is a feedback control where the hypothalamus shed gonadotropic releasing hormone or releasing factor (RF) to stimulate the anterior pituitary gland to release FSH and LH.

LH will stimulate the Leyd cells to secrete testosterone which affects the central nervous system with the growth of bones and muscles in male symbolic appearance and remains of the male glands.

In summary, the level of gonadotropin from the pituitary gland will stimulate the growth and function of the reproductive system while higher levels of androgens will inhibit release secretion factor in Hypothalamus.

One sperm ejaculation will have about 400-500 million sperm and aged 24 hours. If one sperm ejaculation less than 50 million are considered infertile.

Semen collection

Generally, before collecting semen for examination, it is recommended that patients refrain from having sex for at least 3-5 days. If having sex within the period of 3-5 days before being examined, the sperm concentration may be lower than normal. And if abstaining from sex for more than 5 days, there will be an increase in the ageing sperm cell from the epididymis, which will cause the sperm to move less.

Since a single semen examination is less useful in assessing. Therefore, if the semen test results are abnormal. Should be repeated at least 1 time, with the time from the first check for at least 2 weeks.

Semen examination should be done in an examination room that has room temperature or body temperature. As soon as the ejaculation of semen is solidified by protien seminine, which is made from the prostate gland.

To keep the semen locked in the vagina after about 20-30 minutes, the semen becomes liquid again so that the sperm can pass into the cervix. In the event that the semen solidifies longer than usual. And it does not turn into liquid, causing sperm to not pass into the vagina and cause infertility.

If the sperm concentration is less than 20 million per ml, it is called oligospermia. The condition in which the sperm can move less than 50 percent is called asthenozoospermia. And less than 30% of normal morphology is called teratozoospermia.

Table 4. Cutoff reference values in WHO guidelines

Semen characteristics	WHO 2010
Volume (ml)	$\geq 2\%$
pH	≥ 7.2
Sperm count (10^6/ml)	15
Total sperm count (10^6/ml)	$\geq 40\%$
Total motility (% motile)	40
Progressive motility	$\geq 50\%$
Vitality (% alive)	$\geq 75\%$
Viscosity	Normally, semen will coagulate when water ejects and usually liquid within 15-20 minutes.

[f] Strict (Tygerberg) criterion

Source: WHO 2010 guidelines (Organisation, 1999)

Volume; The ranges of normal volume of sexual abstinence of ejaculate after 2–7 days are 2 to 6 ml.

pH; Normal semen pH range is 7.2–8.2 and it will extend with time after ejaculation.

Semen Viscosity; Viscosity measures the struggle of the semen flowing. The normal viscosity is within 15–20 min.

Sperm Concentration; The number of sperm is often reported to be concentrated. (Million sperm per milliliter) including the total number of sperm (Concentration of sperm $\times$ milliliters of semen) in the ejaculate. The diagnosis when it abnormal sperm count are normozoospermia, oligozoospermia, and azoospermia. The lack of sperm in the semen is Azoospermia. The seminal plasma concentration is less than 15 million / mL is namely Oligozoospermia. (Smith, Rodriguez-Rigau, Steinberger, & sterility, 1977)

Motility; The effective way of sperm through the cervical mucosa depends on rapid movement, and the sperm needs to develop forward at least 25 μm / s.

Morphology; The WHO method categorized to classifies irregular shaped sperm based on abnormalities of the head, tail and middle pieces, depends on the presence of sperm that recovers from cervical mucus. and all border patterns are considered abnormal. In general. (Van Waart, Kruger, Lombard, & Ombelet, 2001)

And another studied found that the most fertilization impairment rates happened with morphology scores less than 4 %. (Menkveld, Stander, Kotze, Kruger, & Zyl, 1990)

2.3.3 Factors affecting semen quality

Alcohol and energy beverage consumption

Excessive alcohol consumption is related with a decreased percentage of normal spermatozoa. (Agarwal & Prabakaran, 2005)

Diet and supplement intake

Frequent intake of lipophilic foods like meat products or soymilk may negatively affect semen quality in humans. (Chavarro, Toth, Sadio, & Hauser, 2008) ;meanwhile, some fruits or vegetables may maintain or improve semen quality. (Mendiola et al., 2009). Supplementation with omega3, omega 6 and omega 9 fatty acids, together with vitamin E, for a period of 60 days, increases the quality of ejaculates as it increases semen volume, concentration, and decreases the proportion of abnormal sperm. (Da Rocha, Da Cunha, Ederli, Albernaz, & Quirino, 2009) Also, higher antioxidant intake, such as vitamin C and Vitamin D intake was associated with higher sperm numbers and motility. (Eskenazi et al., 2005)

Exercise

Extreme physical activity may affect the semen concentration, as well as the number of motile and morphologically normal spermatozoa. (Jóźków & Rossato, 2017)

Sleep hour

Excess and short sleep duration and poor sleep quality were associated with reduced semen quality. (Chen et al., 2020) (Hvidt et al., 2020) Also, early bedtime (< 10:30 PM) was more often associated with normal semen quality compared with both regular (10:30 PM-11:29 PM) and late (≥11:30 PM) bedtime. In general sleep duration (7.5–7.99 h) was more often associated with normal semen quality than both short (7.0–7.49 h) and very short (< 7.0 h) sleep duration.

Stress

Stressful life events may be associated with decreased semen quality. Most investigators have reported an association between higher stress levels and lower semen quality. (Hjollund et al., 2004)

Second-hand smoke

High-dose Environmental cigarette smoke (ECS), composed of 89 % sidestream cigarette smoke (SCS) and 11 % the mainstream cigarette smoke (MCS), is able to produce oxidative stress and DNA damage in sperm cells. (La Maestra, De Flora, & Micale, 2014)

Smoking is the confounding factor, the researcher added the questions about smoking data into the questionnaire, such as; How long did you smoke?, How often do you smoke cigarettes?, How many cigarettes do you smoke per day?, Do you live with smoker?

From the article reviewed was shown that smoking duration were negative correlation with the percentage of motility. (Hussein, Algadaa, El Faras, & El Fiky, 2011)

From the article reviewed was shown that smokers compared with never smokers, higher smoking intensity (≥20 cigarettes smoked daily) was related to lower semen volume and total sperm count. (Tang et al., 2019)

From the article reviewed was shown that high-dose environmental cigarette smoke, composed of 89 % sidestream cigarette smoke and 11 % the mainstream cigarette smoke, is able to produce oxidative stress and DNA damage in sperm cells. (La Maestra, De Flora, & Micale, 2014) Furthermore, binary logistic regression analysis will be used to adjust the confounding factors of sperm effects.

2.4 Related Research

Seok-Ju Yoo et al. assess the tobacco at the private and joint incubation of farmers from July to October 2010, the study showed that nicotine level in the farm of tobacco farmers was very high level. (Yoo et al., 2014)

Condorelli RA et al. found that cigarette combustion produces a large number of chemical compounds that may have a negative effect on cigarette smoke on the sperm parameters, the effects on the semen of nicotine, substances contained in the

tobacco plant. And the main components of cigarette smoke which shows that this alkaloids can change the parameters of sperm. (Condorelli et al., 2018)

Parameswari Ranganathan et al. studied about the effects of smoking and the impact on semen parameters. The result shown that the negative cotinine levels have a higher correlation with morphology and rapid movements of infertile smokers than non-smokers. Smokers volunteers have a significant change in the distribution of sperm and cotinine while the negative impact on the motility, morphology and pH of the semen compared to fertility. (Ranganathan, Rao, & Thalaivarasai Balasundaram, 2019)

Lee HJ1 et al. using animal models and human to study about many effects of mother's cigarette smoke during pregnancy on male offspring's sperm count. They use a mouse model to study the effects of cigarette smoke from mothers on the reproductive system. The study found that exposure to cigarette smoke in the uterus decreases sperm count of male offspring. (Lee et al., 2018)

Ibukun Peter Oyeyipo et al. studied about effect of nicotine on sperm characteristics. The studied was conducted with forty rats and twenty-five female rats to study. Male rats were divided into five groups; low dose nicotine and high doses nicotine per body weight while the rats controlled receive normal saline and treated for 30 days. The results showed that sperm motility and the number decreased significantly while the percentage of abnormalities increased significantly in both treatment groups and the reduction in viability and semen volume of the receiving group. That means that nicotine can affect sperm motility, the amount and fertility and the reduced nicotine libido in male rats also. (Oyeyipo, Raji, Emikpe, Bolarinwa, & infertility, 2011)

Mayumi Okuni et al. found that urine cotinine levels in farmers were significantly higher with non-farmers. And farmers who did not wear personal protective equipment (PPE) have more symptoms than those who wear the PPE. In addition, it is found that tobacco growers are getting risk of receiving nicotine toxic when they exposed tobacco leaves. (Onuki et al., 2003)

Tamer M. Said et al. studied the correlation between semen quality and tobacco chewing. The participants were 638 male patients receiving infertility assessments based on the frequency of tobacco chewing habits. They separated

tobacco chewing habit into 3 group (mild, moderate and severe). The result found that the concentration of sperm, percentage of movement, morphology and percentage of viability were significantly higher in the non-violent group compared to the group in the moderate level and in the group with severe level. They end up using tobacco with reduced sperm quality. (Said, Ranga, Agarwal, & sterility, 2005)

Park SJ et al. studied about the cotinine concentration in urine. They measured 5 times with a questionnaire survey. The result shown that significantly cotinine concentration higher during the harvesting season than during the non-harvesting season. (Park, Lim, Lee, Yoo, & work, 2018)

Jing-Bo Dai et al. shown that cigarette smoke contains more than 4000 components, including nicotine, tar, carbonic monoxide. Many studies have reported decrease semen quality, hormonal abnormalities and impaired sperm formation, the growth of sperm, and sperm function in smokers compared with non-smokers. (Dai, Wang, & Qiao, 2015)

Reddy A et al. studied the effect of nicotine on thirteen males with normal fertile nonsmoking were participants and treated with medium alone (control), 10 mm, 5 mm, 1 mm and 0.1 mm nicotine. After incubation sperms were collected at 2, 4, 6 and 24 hrs. The result shown that 0.1 mm without impact, 1 mm significantly reduced sperm motility, 5 mm significantly reduced stroke frequency and 10 mm significantly reduced movement. According to the result, it means nicotine at concentrations of more than or equal 1 mm significantly decreased sperm motion. (Reddy et al., 1995)

Rehana Rehman et al. found that those smoking affects fertility, specific sperm count and normal sperm morphology. They have been conducted 398 participants and categorized the participants into fertile and infertile depending on the sperm cut parameters. After that, the participants in fertile group were 211 and the participants in infertile group were 165. They used Enzyme Immunoassay to determine Serum FSH, LH and Total Testosterone (TT). And using ELISA kits to determine Serum cortisol, adrenaline, superoxide dismutase (SOD), and glutathione peroxidase (GPX). The result was shown that fertile smokers had the mean testosterone level higher than infertile smokers (p-value < 0.05). And also found that

smokers had the total sperm count lower than non-smokers. (Rehman, Zahid, Amjad, Baig, & Gazzaz, 2019)

H. Asare-Anane et al. found that smoking reduced semen quality. They conducted the study from January 2010 to April 2011. The participants in this study were 140 men with the ages range of 18 and 45 years. The measurement tools were semen samples and serum samples. The semen samples were analyzed follow to World Health Organizations guidelines and serum samples were analyzed by enzyme-linked immunosorbent assay including Serum total testosterone, sex hormone binding globulin estradiol, luteinizing hormone, and follicle-stimulating hormone. The result was found that smokers had greater risk of decreasing of semen than nonsmokers. (Asare-Anane et al., 2016)

Jonatan Axelsson et al. found that non-smoking fathers had higher the total number of sperm and the concentration of sperm than the men of the father who smoke. The study was conducted 314 men between age 17–20 years old in Southern Sweden from 2008 to 2010. The measurement tools were maternal serum samples to determine the cotinine using liquid chromatography-tandem mass spectrometry and semen samples to determine volume, sperm concentration, total sperm count, the proportions of morphologically normal and progressively motile sperm follow to World Health Organizations guidelines. The result was shown that men of paternal smoking had a negative relationship between sperm concentration and total sperm count of non-smoker men. Men of the father who smoke had a sperm concentration of 0.41 times and reduced the total sperm count by 0.51 times compared to men of fathers who did not smoke. In addition, men in mothers who smoke (Cotinine > = 15 ng / L) had a lower total sperm count and sperm concentration compared to men in non-smokers mothers. (Axelsson et al., 2018)

Qiuqin Tanga et al. studied about the correlation between tobacco used and semen quality. They conducted 1,631 healthy fertile males in the Nanjing Medical University, China from 2010 to 2016. The measurement tools were semen samples analysis follow to the WHO guidelines. They found that the men who smoke had lower semen volume and total sperm count than the men who did not smoke, especially smokers who had accumulated smoking dose (≥10 packs per year).

Therefore, those who smoke a lot had a low semen volume and total sperm count. (Tang et al., 2019)

CHAPTER III
RESEARCH METHODOLOGY

3.1 Research Design

This study was a longitudinal study to investigate the factors association with semen quality. This study was carried out in male tobacco farmers in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province. The data of this study were collected using the self-administered questionnaires to assess work-related factors, detailed information about tobacco plantation. Semen samples will be analyzed for volume, pH, viscosity, motility, morphology, and sperm count following guidelines and salivary samples will be analyzed for nicotine exposure levels.

Semen samples and saliva samples were collected three times during processing of cultivation tobacco growing, it was collected at first time in the first week of March, 2021 which is the harvesting process, the second time was in the first week of April, 2021 which is the harvesting process and drying process, and to collect the third time in the first week of May, 2021 which is the end of tobacco cultivation.

All participants were provided inform consent, and the protocol was approved by the institutional review boards of the Institutional Review Board of the College of Public Health Sciences, Chulalongkorn University (Code COA No. 115/2564).

3.2 Study period

Male tobacco farmers were recruited from March to May 2022. Data were collected three times during processing of cultivation tobacco growing, the first time was collected in the first week of March 2022 which is the harvesting process, the second time was collected in the first week of April 2022 which is the harvesting process and drying process, and the third time was collected in the first week of May 2022 which is the end of tobacco cultivation.

3.3 Study Population and areas

The populations in the research were male tobacco farmers in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province.

3.4 Research criteria

3.4.1 Inclusion criteria

(1) Age ranged 20 to 40 years old. According to participants selected with male who aged range 20-40 years old because these group are the reproductive age, from the article reviewed that sperm concentration and the proportion of sperm of normal morphology declined after 40 years (Stone, Alex, Werlin, Marrs, & sterility, 2013) Moreover, the most of tobacco farmers in the area are in this age range, so it should represent all age range of tobacco farmers. (Stone, Alex, Werlin, Marrs, & sterility, 2013)

(2) Live in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province and do tobacco farm more than 1 years. Do not plan to move out of the area next following.

(3) Have no history of reproductive abnormal or reproductive disease diagnosed and treated by a doctor, such as hernia, testicular cancer, penile cancer.

(4) Have no history of chronic disease diagnosed and treated by a doctor, such as kidney failure, diabetes, and obesity (A body mass index (BMI) over 30). (Bray, 1992)

(5) Have no history of psychiatric disease diagnosed and treated by a doctor, such as depression, schizophrenia, bipolar disorder, dementia.

3.4.2 Exclusion criteria

(1) Not willing to participate this research.

(2) Cannot participate three times in this study.

3.5 Sampling and Sample size calculation

    3.5.1 Sample size calculation

All male tobacco farmers who registered at Thap Phueng Health Promoting Hospital, Sukhothai Province in season 2020/2021. The sample sizes estimation was calculated by using the formula with the method of calculation as follows;

$$n = \frac{{Z_{\alpha/2}}^2 P(1-P)}{d^2}$$

$$n = \frac{(1.96)^2(0.175)(1-0.175)}{(0.1)^2}$$

$$n = 55.4; \sim 56 \text{ studied samples}$$

where ; n = the estimated sample size,

    $Z_{\alpha/2}$ = the value from normal distribution associated with confidence interval = 1.96 for 95%CI

    α = the level of statistical significance is set as 0.05

    P = The proportion of subjective decrease in total sperm count caused by cigarette smoking (0.175) (Künzle et al., 2003)

    d = The absolute precision required on either side of proportion of the study, the value of 10% was selected.

The total number from calculation was 56 studied samples. However, the researcher added 10 percent for loss to follow-up so, the final number of participants were 62 participants.

    3.5.2 Sampling Technique

    (1) There were nine villages in Thap Phueng Subdistrict, Si Samrong District where represented the most growing tobacco areas and tobacco growing households in Sukhothai Province.

    (2) A purposive selection of nine villages in Thap Phueng Subdistrict were chosen by the place of tobacco cultivation.

(3)   A random selection of the participants was defined by the inclusion criteria, then a systematic sampling technique was used for the selection of male tobacco farmers.
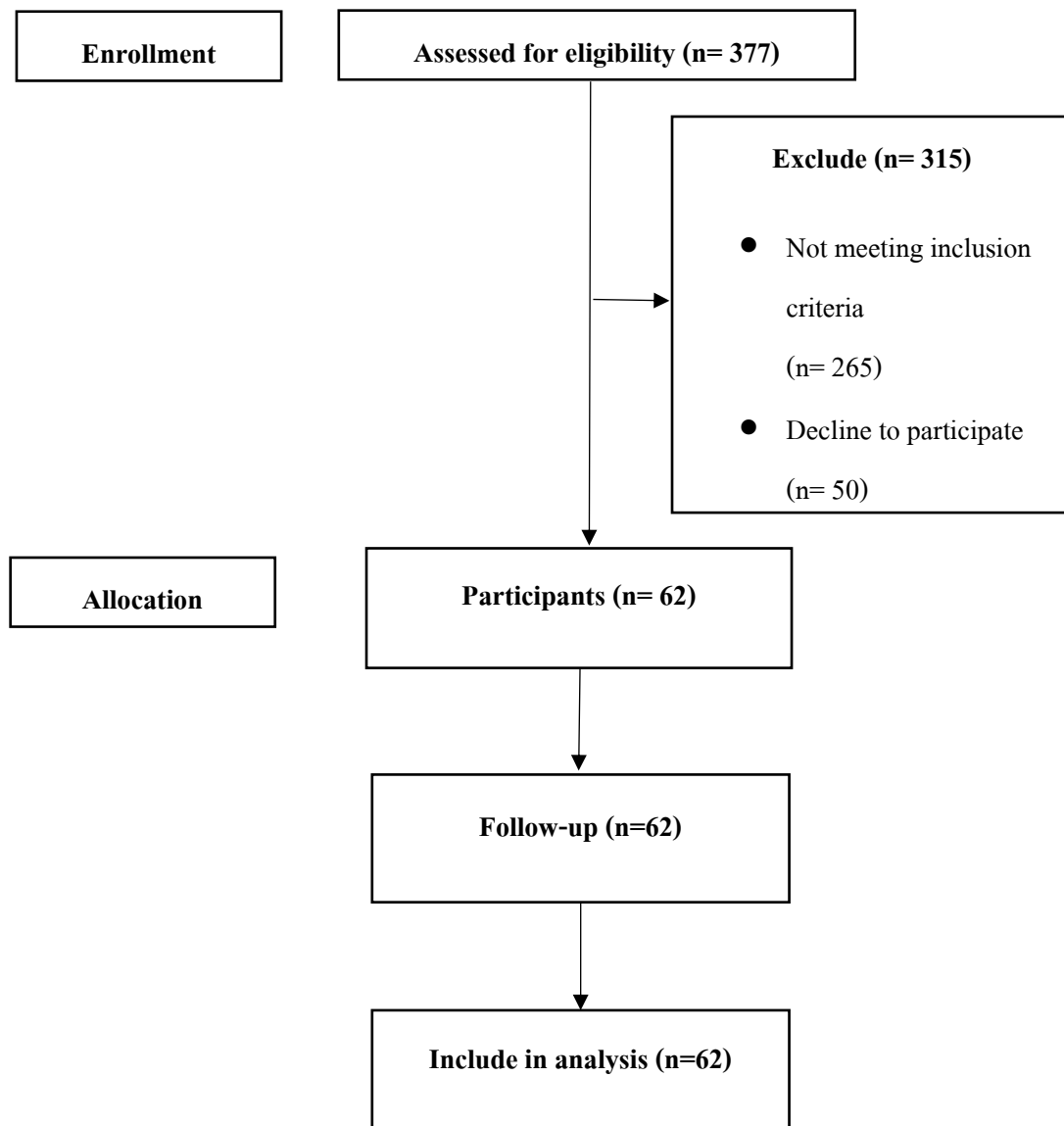
Figure 4. Participants flow chart

3.6 Study procedure

The investigation period was conducted for three time. Semen samples and saliva samples were collected three times during processing of cultivation tobacco growing, it was collected at first time in the first week of March, 2021 which is the

picking first of tobacco plants (T1), the second time was in the first week of April, 2021 which is the picking top of tobacco plants and dry curing of tobacco plants (T2), and to collect the third time in the first week of May, 2021 which is the end of tobacco cultivation.

Table 5. Study procedure

	Picking first of tobacco plants (T1)				Picking top of tobacco plants and dry curing of tobacco plants (T2)				End of cultivation			
Standard	W0	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11
Semen samples	s				s				s			
Salivary cotinine levels by NicAlert[TM] Salivary strip test	x				x				x			

W: weekly

X: Salivary Cotinine test

S: Semen test

1. Seeding   2. Plowing   3. Digging holes for planting   4. Planting

5. Watering   6. Crop maintenance   7. Handpicking   8. Carrying to the agricultural cart

9. Piercing/treading   10. Curing (Air dry in the barn)

Figure 5. Burley tobacco production

Figure 5 shows the steps in tobacco production, starting with the growing period, which usually occurs from November to the end of January. Growing consists of several tasks. It begins with growing the young tobacco plants or seedlings in a greenhouse and then manually transplanting them into the field. Hand tractors and hole digger machines were used for plowing as the soil preparation process and making holes to transplant the young tobacco into them. While the plants grow in the field for several months, crop maintenance is required. This crop maintenance involves watering the plants, application of fertilizer and pesticides, removal of axillary buds and the blooms from the top of the tobacco plant (topping process), and removal of weeds from the field. The postures of the farmers in planting (pegging or setting) the young tobacco plant to the field involve repetitive stooping and bending at the waist. The first crop is ready for harvesting after 60 days, usually from February until May. During this harvesting period, the farmers must start to pick the leaves by hand, beginning from the bottom of the plant and continuing to the top by the end of the harvesting period. The postures of the farmers in this process include stooping and bending the back to the right level for picking the leaves, twisting the wrists for picking the leaves, and carrying leaves with one arm to load them into wagons. Once harvested, the fresh leaves are transported to a barn, where the drying (curing) process takes place. The tasks for the curing (air drying) process include piercing/threading the tobacco leaves onto wooden sticks (60–80 cm or 2–2.5 feet in length). For curing, the loaded sticks are manually lifted onto a wooden rail framework of four to five levels in the barn, beginning at 2 m (6 feet) above the ground floor and continuing to the top of the barn, sometimes 10 m (30 feet) from the ground. After the tobacco leaves are dried, they are taken down from the barn in the reverse process. The dry leaves are stripped from the sticks, loaded into the tobacco press, and compressed into bales; this step is called the baling process. Each bale weighs around 60–70 kilograms, and these are loaded into trucks for shipment.

3.7 Measurement tools

3.7.1 Questionnaire

The data collection was divided into 5 parts:

Part I Socio-demographics characteristics factors (23 questions). There were 23 questions in this part, consisting of age, body weight, height, smoking, living with smokers, alcohol intake, energy beverage intake, diet intake, supplement intake, water intake, exercise and sleep hour.

Part II Stress assessment test (ST5) (5 items). The questions were focused on stress assessment.

Part III Worked-related and Personal protective behaviors (19 questions). There were 19 questions in this part, consisting of experience work with tobacco, current work with tobacco and using Personal protective equipment.

Part IV Salivary cotinine levels by NicAlert™

Salivary cotinine levels were a biomarkers of exposure cotinine measuring by NicAlert™ on tobacco farmers at that time.

Part V Semen test record

Semen samples were a biomarkers of semen quality measuring by semen sample on tobacco farmers at that time.

3.7.2 Salivary cotinine levels

Salivary cotinine levels were a biomarkers of exposure cotinine measuring by NicAlert™ which is based on the principle of enzyme-linked immunosorbent assay (ELISA). In this assay, gold particles coated with monoclonal antibodies were used with a set of thickness traps that allows quantification. The distance that the gold moves on the band was known by clear color changes and provides an accurate cotinine determination in the saliva samples. The test strip was shown seven zones with each zone representing a range of cotinine level.

Stimulated saliva samples were collected from all participants in the morning. First, the participant's mouth was washed with water. The strips were used within 10 minutes after opened.

Procedure

1. Open the package and place the strip on a non-absorbent surface. The plastic was coated instruction card has a marked area.

2. Tear open the saliva collection kit and displace the saliva cone and put on top for the saliva tube container.

3. Place the funnel into the spit tube container and spit into the funnel enough to fill at least 1/2 of the spit container. Then exit the channel.

4. Snap on the top of the saliva tube container and squash 8 drops from the saliva tube, then turn it over at the end of the white cushion of the strip.
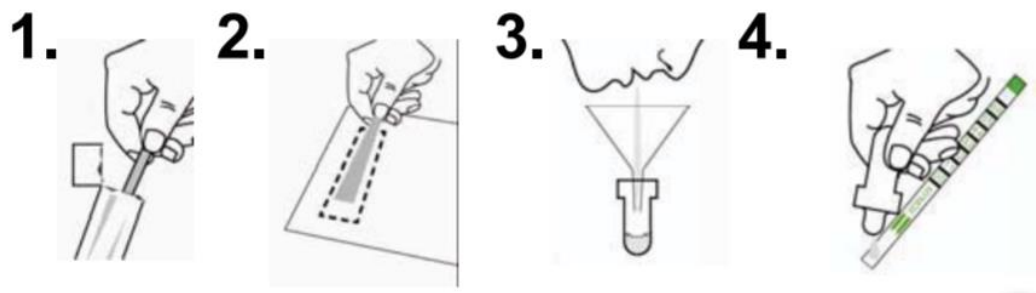
Figure 6. Procedure of testing

The results were read after 15-30 minutes, when the blue stripe disappears or fades significantly. The test results were compared with the cotinine range graph. The strips were read when the lowest band appears in red color as shown in Figure 6.
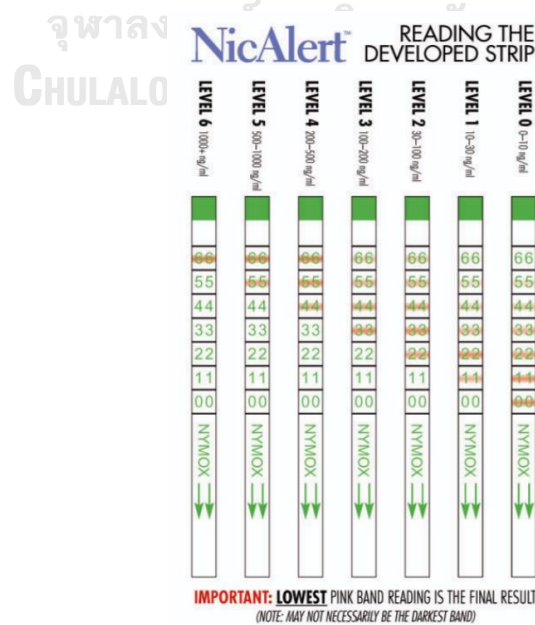
Figure 7. Reading the developed strip

The results of interpretation of the developed test strip as values from 0 to 6 as shown in Table 6.

Table 6. Interpretation of the developed test strip

Level	Cotinine range (ng/ml)
0	0-10
1	10-30
2	30-100
3	100-200
4	200-500
5	500-1000
6	>1000

("Nicalert CE Saliva PI ", 2006)

Salivary cotinine data collection

The salivary samples were conducted once a month in the morning each time, it was collected at first time in the first week of March, 2021, second time was in the first week of April, 2021 and third time was collected in the first week of May, 2021.

    3.7.3 Semen samples

    Semen samples were a biological indicator of sperm quality. It was collected three times, first time in the first week of March, 2021, second time was in the first week of April, 2021 and third time was collected in the first week of May, 2021. Semen samples were analyzed for volume, pH, viscosity, motility, morphology, and sperm count following World Health Organization (WHO) guidelines. (WHO, 2010)

    - Volume. Normal ejaculation volume after 2-7 days of sexual omission is range from 2 to 6 ml.

    - pH. An alkaline coagulation liquid is the main component of semen. It releases from the seminal vesicles flows through the ejaculate tubes. The normal

range of Semen pH is 7.2 to 8.2 and it will be increased depend on the time after ejaculation.

- Viscosity. Viscosity will be measured the resistance of the semen flowing. High viscosity may hinder sperm motility, intensity and sperm antibody coating. Normally, semen will coagulate when water ejects and usually liquid within 15-20 minutes.

- Motility. The effective sperm passing through the cervical mucus depends on rapid movement, that is, the sperm must develop progression at least 25 μm / s forward.

- Morphology. The staining of semen allows the quantitative evaluation of the morphological form of normal and abnormal sperm in the ejaculate. The WHO method categorized to classifies irregular shaped sperm based on abnormalities of the head, tail and middle pieces, depends on the presence of sperm that recovers from cervical mucus. and all border patterns are considered abnormal.

- Sperm count is reported as being concentrated. (Million sperm per milliliter) including the total number of sperm (The concentration of sperm $\times$ milliliter of semen) in the ejaculation. Azoospermia means the absence of sperm in the semen. Oligozoospermia (often referred to as oligospermia) means the plasma semen concentration is less than 15 million per milliliter.

Semen sample collection and delivery

1. The semen samples were collected a minimum of 48 hours but not longer than seven days of sexual abstinence to reduce variability of semen analysis results, the number of days of sexual abstinence should be as constant as possible.

2. The semen samples were collected in a private room near the laboratory.

4. The semen samples were obtained by masturbation and ejaculated into a lean, wide-mouthed container made of plastic and sterile container.

7. The semen samples were protected from extremes of temperature (less than 20 ºC and more than 40 ºC) during transport to the laboratory.

8. The containers were adequately labelled with subject's name and with the date and time of collection.

9. The semen samples were analyzed by the laboratory technician within 1 hour.

3.8 Validity and Reliability

3.8.1 Validity test of the instrument

The validity of questionnaire was checked by three experts in the field of the occupational health and environmental.

3.8.2 Reliability test of the instrument

After the questionnaire was revised, the pilot test was done in 30 study population from male tobacco farmers in other village where was tobacco farm for reliability test of the questionnaire. Cronbach's alpha coefficient was used to measure the reliability of the questionnaire, it must have a value of coefficient above 0.7 (DeVellis, 2016) that was considered as satisfactory.

The reliability test results of the questionnaires for 1 part consisted of work-related factor had a value of coefficient above 0.7.

3.8.3 Index of Congruence: (IOC)

Content validity testing on the data collection tools by using the data collection tools to check the content validity by the experts in the field of occupational health and environmental, after passing the examination of the experts, the content validity was evaluated by using the Index of Congruence: IOC, which was following scores;

Consistency that score is +1 point

Not sure score that score is 0 point

Inconsistency that score is -1 point

Then combine the points of the three experts in each item to find the consistency of the value from the formula with the following criteria; Giving a score of +1 point means the experts were sure that the questions were consistent with the objectives.

Giving a score of 0 points means the experts were not sure that the questions were consistent with the objectives.

Giving a score of -1 point means the experts were sure that the questions were inconsistent with the objectives.

Therefore, it can be concluded that the standard value of the consistency index of congruence (IOC) of the question that had the value of the validity of the content must have an IOC of 0.50 - 1.00, but if the question had content validity with an IOC of less than 0.50, it means that the question was still unavailable and the question must be to improve.(Keanchunbai, 2017).

3.9 Data Analysis

Relevant statistics included;

3.9.1 Descriptive statistics comprised of frequency, percentage, mean, and standard deviation were used for the analysis of socio-demographic factors and work-related factors and personal protective behavior.

3.9.2 Inferential statistics

(1) The Kolmogorov-Smirnov test was used for testing of normality.

(2) Friedman Test was used to compare the salivary cotinine levels between three season periods.

(3) Repeated-measure ANOVA was used to compare the semen quality between three season periods.

(4) Binary logistic regression was used to analyze an association between individual factors, work-related factors with salivary cotinine levels and semen quality.

The data analysis is shown in table **7**.

Table 7. Method of data analysis

Objectives	Main Variables	Statistic(s) used
To explore the association between individual factors, work-related factors with semen quality among male tobacco farmers in Sukhothai Province, Thailand.	- All of independent variables - All of dependent Variables	- Descriptive Statistics - Multiple linear regression
To explore the association between individual factors, work-related factors with salivary cotinine levels	- All of independent variables - All of dependent	- Descriptive Statistics - Binary logistic regression

Objectives	Main Variables	Statistic(s) used
among male tobacco farmers in Sukhothai Province, Thailand.	Variables	
To explore the association between salivary cotinine levels with semen quality among male tobacco farmers in Sukhothai Province, Thailand.	- All of independent variables - All of dependent Variables	- Descriptive Statistics - Binary logistic regression
To evaluate salivary cotinine levels among male tobacco farmers in Sukhothai Province, Thailand.	All of dependent Variables	Descriptive Statistics
To evaluate the semen quality among male tobacco farmers in Sukhothai Province, Thailand.	All of dependent Variables	Descriptive Statistics
To evaluate the salivary cotinine levels different between the season periods among male tobacco farmers in Sukhothai Province, Thailand.	All of dependent Variables	Friedman Test
To evaluate the semen quality different between the season periods among male tobacco farmers in Sukhothai Province, Thailand.	All of dependent Variables	- Repeated-measure ANOVA

3.10 Ethical consideration

3.10.1 This study was approved by the research ethics review committee for research Involving Human group I, Chulalongkorn University (Code COA No. 115/2564).

3.10.2 This study was approved by Sukhothai Provincial Public Health Office.

3.10.3 The letters were written to the head of village to obtain permission and cooperation for data collection.

3.10.4 Informed consent and information sheet were secured from the study.

3.11 Expected Outcomes and Benefit

3.11.1 The male tobacco farmers understood the consequences of nicotine exposure in tobacco farmers.

3.11.2 To increase public awareness about the health adverse effects of tobacco plantations.

3.11.3 To recommendation for personal protection of working in tobacco farms.

3.11.4 To understand characteristics of semen quality in male tobacco farmers.

3.12 The study schedule

The estimate duration of the study was about 15 months between August 2021 to October 2022. The details of the time estimate were shown in table 8.

Table 8. The study schedule

Activities	2021					2022									
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct
Literature reviews	√	√													
Proposal writing		√	√												
Proposal exam and presentation					√										
Ethical approval						√	√	√							
Data Collecting							√	√		√					
Data Analysis											√	√			
Report writing and presentation															√

3.13 Budget

The total of this study was approximately 316,500 baht. Detail of the budget was shown in table 9.

Table 9. Budget of the study

Category	Unit	Cost (Thai Baht)
Material Cost		
1. Questionnaire preparation	62 x 50 Baht	3,100
2. Salivary cotinine sample	62 x 3 x 1,000 Baht	186,000
3. Semen samples	62 x 3 x 300 Baht	55,800
4. Report preparation	1 x 800 Baht	800
Allowance and utility expense		
1. Travelling expense	5 weeks x 2,000	10,000
2. Travelling expense of participants	62 x 3 x 300 Baht	55,800
3. Miscellaneous		5,000
Total		**316,500**

CHAPTER IV
RESULTS

This study was a longitudinal study. Male tobacco farmers who lived in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province recruited 62 male tobacco farmers fulfilled the criteria for selection and volunteered to participate in this study. The questionnaires were used to interview the male tobacco farmers about general characteristics, work-related factors and detailed information about tobacco plantation. Semen quality including volume, pH, viscosity, motility, morphology, and sperm count were analyzed using semen. Salivary samples were analyzed for nicotine exposure levels. The results were presented into six parts as follows:

4.1. Demographic characteristics of male tobacco farmers

4.2 Assessment of stress assessment (ST5) of male tobacco farmers

4.3 Assessment of exposure to nicotine and personal protective behaviors of male tobacco farmers

4.4 Assessment of salivary cotinine levels and to evaluate the salivary cotinine levels different between the season periods.

4.5 Assessment of semen quality and to evaluate the semen quality different between the season periods.

4.6 The association of variable

4.6.1 The association between individual factors, work-related factors with salivary cotinine levels

4.6.2 The association between individual factors, work-related factors and salivary cotinine levels with semen quality.

4.1 Demographic characteristics of male tobacco farmers

There were 62 male tobacco farmers who participated in the questionnaire interview. As regards demographic characteristics of the farmers, the average age of male tobacco farmers was 35 years old. The Body Mass Index (BMI) of male tobacco farmers was classified into 3 groups. The major Body Mass Index (BMI) of male tobacco farmers was normal weight (72.6%) and the rest was under weight (4.8%),

while the average Body Mass Index (BMI) was 23.26 kg/m^2. As for history of smoking, 50.0% of male tobacco farmers smoked cigarettes. In addition, most of them smoked less than 10 years (25.8%), smoked everyday (41.9%) and, the number of cigarettes smoked was 10 to 20 cigarettes/day (32.3%). The history of alcohol drinking, 69.4% of male tobacco farmers drank alcohol, most of them drank with 5 or more times a week (55.8%). The history of energy beverage consumption, 90.3% had history of energy beverage consumption, and most of them consumed 5 or more times a week (67.9%). The history of 5 food groups consumption, 67.7% of male tobacco farmers had history of 5 food groups consumption, most of them consumed 5 or more times a week (64.5%). The history of supplements consumption, 95.2% of male tobacco farmers did not consume the supplements. The history of water drinking, 87.1% of male tobacco farmers drank more than 8 glasses per day. Moreover, haft of male tobacco farmers did not exercise (61.3%), 59.7% of male tobacco farmers had sleeping hour more than 8 hours. The findings regarding demographic characteristics of the male tobacco farmers are summarized in Table 10 below.

The descriptive characteristics of 62 male tobacco farmers are presented in Table 10.

Table 10. Demographic characteristics among male tobacco farmers (n = 62)

Characteristics	n	%
Age group (years)		
20-24	12	19.4
25 -29	2	3.2
30-34	6	9.7
≥ 35	42	67.7
Mean= 35.22 ,S.D> = 6.698 , Min= 20 , Max= 40		
Body Mass Index (BMI)		
Underweight (< 18.5)	3	4.8
Normal weight (18.5-24.9)	45	72.6

Characteristics	n	%
Over weight (25.0-29.9)	14	22.6
Mean= 23.26 ,S.D> = 2.872 , Min= 16.50 , Max= 29.95		
Smoking		
No	25	40.3
Yes	31	50.0
Living with smokers		
No	22	35.5
Yes	40	64.5
Alcohol drinking		
No	19	30.6
Yes	43	69.4
Energy beverage consumption		
No	6	9.7
Yes	56	90.3
Supplements consumption		
No	59	95.2
Yes	3	4.8
Water drinking (glasses per day)		
< 8	8	12.9

Characteristics	n	%
≥ 8	54	87.1
Exercise		
No	38	61.3
Yes	24	38.7
Sleeping hours		
< 8 hours	25	40.3
≥ 8 hours	37	59.7

4.2 Assessment of stress assessment (ST5) of male tobacco farmers

As shown in Table 11, most of male tobacco farmers were less stressful (72.1%), moderate stressful (24.6%) and the rest was very stressful (3.3%).

Table 11. Stress assessment (ST5) among male tobacco farmers (n = 62)

Stress levels	n	%
Less stressful.	44	72.1
Moderate stressful.	15	24.6
Very stressful.	2	3.3
Mean = 2.15, S.D. = 2.386, Max = 8, Min = 0		

4.3 Assessment of exposure to nicotine and personal protective behaviors of male tobacco farmer

Assessment of exposure to nicotine and personal protective behaviors of 62 male tobacco farmer is presented in Table 12 and 13.

Table 12 presents the assessment of exposure to nicotine, most of tobacco farmers had 2 to 3 times per years of growing seasons (71.0%). 87.1% of male tobacco farmers

were owner and do it themselves. The history of male tobacco farmers with start working as an agricultural worker/farmer, most of male tobacco farmers start working were less than 20 years old (77.4%). The major of male tobacco farmers had experience with tobacco plantation more than 20 years (64.5%). In addition, most of them did all processing for tobacco plantation (90.3%). The processing for tobacco plantations were 4 processing, the experience with seeding growing, most of male tobacco farmers worked 4-7 days per week (87.1%). 69.4% of male tobacco farmers worked less than 8 hours per day, the experience with transplanting to the tobacco field, most of male tobacco farmers worked 4-7 days per week (79.0%). 59.7% of male tobacco farmers worked more than 8 hours per day, the experience with picking tobacco leaves, most of male tobacco farmers worked 4-7 days per week (90.3%). 83.9% of male tobacco farmers worked less than 8 hours per day, the experience with drying tobacco leaves, most of male tobacco farmers worked 4-7 days per week (83.9%). 66.1% of male tobacco farmers worked less than 8 hours per day. The findings regarding assessment of exposure to nicotine of the male tobacco farmers are summarized in Table 12 below.

Table 12. Assessment of exposure to nicotine among male tobacco farmers (n = 62)

Assessment of exposure to nicotine	n	%
Growing seasons (times per years)		
less than 2	15	24.2
2-3	44	71.0
more than 3	3	4.8
Owner of the farm		
Own and do it	54	87.1
Own and hire others to do	1	1.6
Rent and do it	5	8.1
Rent and hire others to do	2	3.2

Assessment of exposure to nicotine	n	%
Start working as an agricultural worker/farmer		
less than 20 years	48	77.4
21-30 years	13	21.0
31-40 years	1	1.6
Experience with tobacco plantation (years)		
1-5	5	8.1
6-10	13	21.0
11-15	2	3.2
16-20	2	3.2
> 20	40	64.5
Processing for tobacco plantation		
Seeding growing	5	8.1
Transplanting to the tobacco field	1	1.6
All processing	56	90.3
Experience with seeding growing (days per month)		
2 times a month or less	5	8.1
1 day a week or less	1	1.6
2-3 days a week	2	3.2
4-7 days a week	54	87.1

Assessment of exposure to nicotine	n	%
Experience with seeding growing (hours per day)		
< 8	43	69.4
≥ 8	19	30.6
Experience with transplanting to the tobacco field (days per month)		
2 times a month or less	6	9.7
1 day a week or less	1	1.6
2-3 days a week	6	9.7
4-7 days a week	49	79.0
Experience with transplanting to the tobacco field (hours per day)		
< 8	25	40.3
≥ 8	37	59.7
Experience with picking tobacco leaves (days per month)		
2 times a month or less	1	1.6
1 day a week or less	2	3.2
2-3 days a week	4	6.5
4-7 days a week	56	90.3

Assessment of exposure to nicotine	n	%
Experience with picking tobacco leaves (hours per day)		
< 8	52	83.9
≥ 8	10	16.1
Experience with drying tobacco leaves (days per month)		
2 times a month or less	1	1.6
1 day a week or less	0	0.0
2-3 days a week	9	14.5
4-7 days a week	52	83.9
Experience with drying tobacco leaves (hours per day)		
< 8	41	66.1
≥ 8	21	33.9
Pesticides applying (time per growing season)		
Every month	29	46.8
More often than every month	33	53.2
Pesticides applying (day per month)		
Never	1	1.6
1-4 days	50	80.6
5-10 days	6	9.7

Assessment of exposure to nicotine	n	%
More than 10 days	5	8.1
Duration of pesticides applying		
1-5 years	8	12.9
6-10 years	11	17.7
11-15 years	2	3.2
16-20 years	2	3.2
> 20 years	39	62.9

Table 13 presents the personal protective behaviors among male tobacco farmers usage during work with tobacco processing. Most of the male tobacco farmers reported that they always used PPE (boots or closed shoes and long-sleeved shirt). However, 35.5%, 43.5% and 59.7% of the male tobacco farmers had never worn a raincoat, chemical-resistant rubber gloves and a plastic apron, respectively. In addition, most of tobacco farmers had shower and clean body immediately after work (95.2%), immediately take a bath and change clothes when finished job (85.5%) and work clothes washed separate with other clothes (77.4%), respectively.

Table 13. Personal protective behaviors among male tobacco farmers (n = 62)

Personal protective behaviors	Male tobacco farmer		
	Never	**Sometimes**	**Always**
Immediately take a bath and change clothes when finished job	1 (1.6)	8 (12.9)	53 (85.5)
Work clothes washed separate with other clothes	10 (16.1)	4 (6.5)	48 (77.4)
Shower and clean body immediately after	1 (1.6)	2 (3.2)	59 (95.2)

Personal protective behaviors	Male tobacco farmer		
	Never	**Sometimes**	**Always**
work			
Clothes wet while working	0 (0)	10 (16.1)	52 (83.9)
Chemical-resistant rubber gloves	27 (43.5)	13 (21.0)	22 (35.5)
Boots or closed shoes	5 (8.1)	6 (9.7)	51 (82.3)
Long-sleeved shirt	0 (0)	0 (0)	62 (100)
Raincoat	22 (35.5)	22 (35.5)	18 (29.0)
Plastic apron	37 (59.7)	19 (30.6)	6 (9.7)

4.4 Assessment of salivary cotinine levels and to evaluate the salivary cotinine levels different between the season periods

Table 14 shows the distribution of salivary cotinine levels in male tobacco farmers by time of testing. All the testing in three times, in the test of T1, T2 and T3 was found that in T2 have number of saliva cotinine exposure on level 2,3 and 4 more than T1 and T3 that measure by NCTS strip test. Test 1, The most of male tobacco farmers on level 0 were thirty-eight persons (61.3%), level 1 were eleven persons (17.7%), level 2 were eight persons (12.9%) and level 3 were fifth persons (8.1%), respectively. Test 2, The most of male tobacco farmers on level 0 were twenty-fifth persons (40.3%), level 3 were twenty persons (32.3%), level 2 were nine persons (14.5%), level 4 were fifth persons (8.1%) and level 1 were three persons (4.8%), respectively. Test 3, The most of male tobacco farmers on level 0 were fifty-nine persons (95.2%), level 1were two persons (32.3%), and level 2 were one person (1.6%), respectively.

Table 14. Distribution of salivary cotinine levels in male tobacco farmers by time of testing (n = 62)

Level	Cotinine concentration (ng/ml)	T1 n (%)	T2 n (%)	T3 n (%)
0	0-10	38 (61.3)	25 (40.3)	59 (95.2)
1	10-30	11 (17.7)	3 (4.8)	2 (3.2)
2	30-100	8 (12.9)	9 (14.5)	1 (1.6)
3	100-200	5 (8.1)	20 (32.3)	0 (0.0)
4	200-500	0 (0.0)	5 (8.1)	0 (0.0)

Note: T = time of testing.

Table 15 present the comparison of salivary cotinine levels on male tobacco farmers in three time of season periods, the result was shown that the salivary cotinine levels of male tobacco farmers in picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly higher than those of male tobacco farmers in picking first of tobacco plants (T1) and male tobacco farmers in the end of cultivation season period (T3) at p-value < 0.05 ($X^2 = 60.18$). The salivary cotinine levels are significant different between picking first of tobacco plants (T1), picking top of tobacco plants and dry curing of tobacco plants (T2) and end of cultivation season period (T3).

Table 15. The comparison of saliva cotinine levels in male tobacco farmers at picking first of tobacco plants (T1), picking top of tobacco plants and dry curing of tobacco plants (T2) and end of cultivation season period (T3) (n= 62)

Parameter	T1 Median Rank	T2 Median Rank	T3 Median Rank	X^2
Salivary cotinine levels	1.94	2.56	1.51	60.18[a]

Note: T = time of testing

[a] P < 0.05 was considered significant using Friedman Test.

4.5 Assessment of semen quality and to evaluate the semen quality different between the season periods

Table 16 shows the distribution of semen quality in male tobacco farmers by time of testing. Test 1, The most of male tobacco farmers had normal semen motility 40 (64.5%), had normal morphology 55 (87.5%), and had normal sperm count 31 (50.0%). Test 2, The most of male tobacco farmers had abnormal semen motility 32 (51.6%), had normal morphology 35 (56.5%), and had abnormal sperm count 44 (71.0%). T Test 3, The most of male tobacco farmers had normal semen motility 50 (80.6%), had normal morphology 50(80.6%), and had normal sperm count 35 (56.5%).

Table 16. Distribution of semen quality in male tobacco farmers by time of testing (n = 62)

Semen parameters	T1		T2		T3	
	Normal n (%)	**Abnormal** n (%)	**Normal** n (%)	**Abnormal** n (%)	**Normal** n (%)	**Abnormal** n (%)
Motility	40(64.5%)	22(35.5%)	30(48.4%)	32(51.6%)	50(80.6%)	12(19.4%)
Morphology	55(87.5%)	7(11.3%)	35(56.5%)	27(43.5%)	50(80.6%)	12(19.4%)
Sperm count	31(50.0%)	31(50.0%)	18(29.0%)	44(71.0%)	35(56.5%)	27(43.5%)

Note: T = time of testing.

In conclusion from Table 17, the measure of mean differences at three season periods in the male tobacco farmers showed that semen volume, pH, motility, morphology and sperm count in the picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly lower than those in the picking first of tobacco plants (T1) and those in the end of cultivation season period (T3) at p-value < 0.05.

it appears apparent that results of the repeated measure ANOVA reveal how there was an overall significant difference in semen parameters between three season periods, meaning the effect of time was significantly different.

Interpretation of semen parameter mean differences at three season periods. It can be described as follows, the picking top of tobacco plants and dry curing of tobacco plants (T2) the picking first of tobacco plants (T1)

- Semen volume in the picking top of tobacco plants and dry curing of tobacco plants (T2) was significantly lower than the picking first of tobacco plants (T1) and the end of cultivation season period (T3) (p-value < 0.001).

- Semen pH in the picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly lower than the end of cultivation season period (T3) (p-value = 0.007).

- Semen viscosity in the picking top of tobacco plants and dry curing of tobacco plants (T2) was significantly higher than the picking first of tobacco plants (T1) and the end of cultivation season period (T3) (p-value < 0.001).

- Semen motility in the picking top of tobacco plants and dry curing of tobacco plants (T2) was significantly lower than the picking first of tobacco plants (T1) and the end of cultivation season period (T3) (p-value < 0.001).

- Semen morphology in the picking top of tobacco plants and dry curing of tobacco plants (T2) was significantly lower than the picking first of tobacco plants (T1) and the end of cultivation season period (T3) (p-value < 0.001).

- Sperm count in the picking top of tobacco plants and dry curing of tobacco plants (T2) was significantly lower than the picking first of tobacco plants (T1) and the end of cultivation season period (T3) (p-value < 0.001).

Table 17. The comparison of the sperm parameters in male tobacco farmers at the picking first of tobacco plants (T1), the picking top of tobacco plants and dry curing of tobacco plants (T2) and the end of cultivation season period (T3) (n= 62)

Parameter	T1	T2	T3
	Mean ± SD	Mean ± SD	Mean ± SD
Volume (ml)	$2.88 \pm 0.08^{b,c}$	$2.34 \pm 0.05^{a,c}$	$3.54 \pm 0.06^{a,b}$
pH	7.15 ± 0.25	7.119 ± 0.19^{c}	7.20 ± 0.17^{b}
Viscosity (cm)	$23.45 \pm 7.88^{b,c}$	$29.01 \pm 7.96^{a,c}$	$21.85 \pm 7.37^{a,b}$
Motility (%)	64.51 ± 11.37^{b}	$55.80 \pm 10.13^{a,c}$	64.51 ± 10.31^{b}
Morphology (%)	$66.20 \pm 7.45^{b,c}$	$56.85 \pm 6.54^{a,c}$	$63.30 \pm 7.30^{a,b}$
Sperm count (10^{6} /ml)	37.99 ± 1.36^{b}	$29.98 \pm 1.20^{a,c}$	39.45 ± 1.27^{b}

Note: Values are expresses as mean ± standard deviation. P < 0.05 was considered significant using Repeated-measure ANOVA compared to: [a]time1; [b] time2; [c]time3, T = time of testing.

4.6 The association of variable

4.6.1 The association between individual factors, work-related factors with salivary cotinine levels

As shown in table 18, based on results from binary logistic regression predicting salivary cotinine levels in male tobacco farmers at the picking first of tobacco plants (T1), age was significantly reduced salivary cotinine levels by an average of 8% (OR = 0.92, 95% CI 0.85-0.99, p = 0.045). Moreover, alcohol intake was significantly reduced salivary cotinine levels by an average of 77% (OR = 4.43, 95% CI 1.41-13.93, p = 0.011). Then, after adjusting for age and smoking, we found that age (OR = 0.92, 95% CI = 0.85-0.99, p = 0.044) and the alcohol intake (OR=5.93, 95% CI = 1.66-21.21, p = 0.006) were independently associated with salivary cotinine levels.

Table 18. Binary logistic regression predicting salivary cotinine levels[a] in male tobacco farmers at the picking first of tobacco plants (T1) (n=62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age[b]	0.92	0.85-0.99	0.045*	0.92	0.85-0.99	0.044*
Body mass index	2.25	0.72-7.01	0.162	3.07	0.88-10.67	0.077
Smoking	1.50	0.48-4.72	0.489	1.38	0.41-4.67	0.602
Living with smoker	1.15	0.39-3.34	0.792	1.23	0.36-4.23	0.743
Alcohol intake	4.43	1.41-13.93	0.011*	5.93	1.66-21.21	0.006*
Water intake	0.94	0.20-4.37	0.940	0.71	0.14-3.51	0.673
Stress	1.11	0.36-3.48	0.86	1.24	0.38-4.05	0.726
Working experience	2.89	0.31-26.55	0.349	2.69	0.29-25.17	0.387
Wearing gloves	0.61	0.22-1.72	0.350	0.62	0.21-1.86	0.392
Wearing raincoat	0.63	0.21-1.89	0.410	0.61	0.19-1.90	0.390
Wearing plastic apron	0.62	0.21-1.78	0.370	0.67	0.22-2.05	0.481
Day spent working	1.58	0.37-6.82	0.539	1.77	0.36-8.68	0.482
Hour spent working	1.23	0.41-3.69	0.715	1.86	0.56-6.19	0.313

Note: [a]=Saliva cotinine level (Low expose=0, High expose=1), [b] = continuous data
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in table 19, based on results from binary logistic regression predicting salivary cotinine levels in male tobacco farmers at the picking top of tobacco plants and dry curing of tobacco plants (T2), there were no significant associations between factors with salivary cotinine levels.

Table 19. Binary logistic regression predicting salivary cotinine levels[a] in male tobacco farmers at the picking top of tobacco plants and dry curing of tobacco plants (T2) (n=62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age[b]	0.95	0.88-1.03	0.227	0.95	0.88-1.03	0.241
Body mass index	1.92	0.58-6.36	0.286	3.17	0.82-12.17	0.093
Smoking	2.61	0.92-7.43	0.073	2.60	0.90-7.50	0.077
Living with smoker	1.09	0.33-3.53	0.892	1.18	0.35-3.92	0.792
Alcohol intake	1.11	0.37-3.33	0.849	1.04	0.33-3.27	0.951
Water intake	0.49	0.83-2.43	0.353	0.46	0.08-2.68	0.390
Stress	1.11	0.36-3.48	0.86	1.24	0.38-4.05	0.726
Working experience	1.19	0.22-6.48	0.837	1.89	0.19-18.92	0.588
Wearing gloves	0.71	0.25-2.02	0.515	0.75	0.24-2.19	0.545
Wearing raincoat	0.77	0.27-2.58	0.638	0.81	0.27-2.46	0.709
Wearing plastic apron	0.78	0.28-2.17	0.628	0.78	0.26-2.28	0.643
Day spent working	1.55	0.29-8.36	0.613	2.29	0.37-14.12	0.374
Hour spent working	1.71	0.39-7.37	0.471	1.84	0.39-8.50	0.434

Note: [a]=Saliva cotinine level (Low expose=0, High expose=1), [b] = continuous data
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in table 20, based on results from binary logistic regression predicting salivary cotinine levels in male tobacco farmers at the end of cultivation season period (T3), there were no significant associations between factors with salivary cotinine levels. According to some variable had N/A because most of participants' salivary cotinine levels were on low expose group.

Table 20. Binary logistic regression predicting salivary cotinine levels[a] in male tobacco farmers at the end of cultivation season period (T3) (n=62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age[b]	1.19	0.79-1.77	0.398	1.16	0.80-1.67	0.432
Body mass index	5.87	0.49-69.42	0.160	4.92	0.41-59.31	0.210
Smoking	N/A	N/A	N/A	N/A	N/A	N/A
Living with smoker	N/A	N/A	N/A	N/A	N/A	N/A
Alcohol intake	N/A	N/A	N/A	N/A	N/A	N/A
Water intake	N/A	N/A	N/A	N/A	N/A	N/A
Stress	1.31	0.11-15.49	0.829	1.18	0.10-14.29	0.894
Working experience	N/A	N/A	N/A	N/A	N/A	N/A
Wearing gloves	N/A	N/A	N/A	N/A	N/A	N/A
Wearing raincoat	0.91	0.08-10.58	0.936	0.95	0.08-11.44	0.970
Wearing plastic apron	N/A	N/A	N/A	N/A	N/A	N/A
Day spent working	N/A	N/A	N/A	N/A	N/A	N/A
Hour spent working	2.78	0.23-33.95	0.424	3.00	0.20-44.36	0.424

Note: [a]=Saliva cotinine level (Low expose=0, High expose=1), [b] = continuous data
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

4.6.2 The association between individual factors, work-related factors and salivary cotinine levels with semen quality.

As shown in Table 21, based on results from binary logistic regression predicting semen motility among male tobacco farmers in the picking first of tobacco plants (T1), working experience was significantly reduced semen motility by an average of 84% (OR = 0.16, 95% CI 0.03-0.98, p = 0.048). Then, after adjusting for age and smoking, we found that working experience (OR = 0.16, 95% CI = 0.03-0.99, p = 0.048) was independently associated with semen motility.

Table 21. Binary logistic regression predicting semen motility[a] among male tobacco farmers in the picking first of tobacco plants (T1) (n=62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	1.13	0.33-3.87	0.842	1.13	0.33-3.87	0.841
Smoking	1.00	0.35-2.83	1.000	1.00	0.35-2.85	0.994
Stress	1.97	0.63-6.17	0.245	1.98	0.62-6.27	0.248
Working experience	0.16	0.03-0.98	0.048*	0.16	0.03-0.99	0.048*
Day spent working	1.11	0.19-6.61	0.908	1.13	0.18-7.06	0.895
Hour spent working	3.38	0.84-13.63	0.088	3.44	0.83-14.24	0.088
Wearing gloves	1.16	0.39-2.22	0.839	1.11	0.38-3.21	0.847
Salivary cotinine levels	1.15	0.39-3.34	0.792	1.22	0.39-3.78	0.727

Note: [a]= Semen motility (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 22, based on results from binary logistic regression predicting semen morphology among male tobacco farmers in the picking first of tobacco plants (T1), there were no significant associations between factors with semen morphology. According to some variable had N/A because most of participants' semen morphology were on normal group.

Table 22. Binary logistic regression predicting semen morphology[a] among male tobacco farmers in the picking first of tobacco plants (T1) (n=62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	2.05	0.23-18.53	0.523	2.02	0.22-18.36	0.531
Smoking	0.72	0.15-3.54	0.689	0.74	0.15-3.62	0.706
Stress	1.07	0.19-6.10	0.942	0.95	0.16-5.69	0.959
Working experience	N/A	N/A	N/A	N/A	N/A	N/A
Day spent working	N/A	N/A	N/A	N/A	N/A	N/A
Hour spent working	N/A	N/A	N/A	N/A	N/A	N/A
Wearing gloves	0.52	0.11-2.34	0.415	0.49	0.09-2.45	0.384
Salivary cotinine levels	0.60	0.11-3.37	0.562	0.67	0.11-4.11	0.669

Note: [a]= Semen morphology (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 23, based on results from binary logistic regression predicting sperm count among male tobacco farmers in the picking first of tobacco plants (T1), there were no significant associations between factors with sperm count.

Table 23. Binary logistic regression predicting sperm count[a] among male tobacco farmers in the picking first of tobacco plants (T1) (n=62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	0.84	0.26-2.67	0.767	0.83	0.26-2.68	0.759
Smoking	0.88	0.33-2.38	0.800	0.87	0.32-2.37	0.791
Stress	1.63	0.53-5.05	0.395	1.65	0.53-5.17	0.390
Working experience	0.75	0.27-2.14	0.596	0.65	0.13-3.35	0.603
Day spent working	2.15	0.36-12.69	0.399	2.09	0.34-12.89	0.427
Hour spent working	1.00	0.26-3.87	1.000	1.05	0.27-4.15	0.945
Wearing gloves	0.47	0.17-1.32	0.152	0.47	0.17-1.33	0.155
Salivary cotinine levels	1.31	0.47-3.66	0.602	1.27	0.43-3.76	0.668

Note: [a]= Sperm count (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 24, based on results from binary logistic regression predicting semen motility among male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2), there were no significant associations between factors with semen motility.

Table 24. Binary logistic regression predicting semen motility[a] among male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2) (n= 62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	1.29	0.41-4.16	0.660	1.32	0.41-4.23	0.645
Smoking	1.29	0.48-3.52	0.612	1.31	0.48-3.56	0.599
Stress	1.08	0.35-3.29	0.898	1.09	0.35-3.37	0.885
Working experience	0.63	0.22-1.79	0.384	0.16	0.02-1.43	0.101
Day spent working	1.07	0.19-5.78	0.934	1.23	0.22-6.96	0.817
Hour spent working	1.50	0.38-5.94	0.564	1.40	0.35-5.68	0.635
Wearing gloves	0.69	0.25-1.93	0.481	0.68	0.24-1.89	0.460
Salivary cotinine levels	0.97	0.35-2.69	0.960	0.95	0.33-2.73	0.918

Note: [a]= Semen motility (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 25, based on results from binary logistic regression predicting semen morphology among male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2), there were no significant associations between factors with semen morphology.

Table 25. Binary logistic regression predicting semen morphology[a] among male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2) (n= 62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	0.85	0.26-2.72	0.780	0.84	0.26-2.71	0.772
Smoking	0.88	0.32-2.39	0.798	0.87	0.32-2.39	0.789
Stress	1.69	0.55-5.19	0.361	1.71	0.55-5.32	0.357
Working experience	0.50	0.17-1.44	0.198	0.22	0.04-1.32	0.098
Day spent working	4.33	0.48-39.51	0.194	4.33	0.46-40.92	0.201
Hour spent working	0.84	0.21-3.34	0.805	0.88	0.22-3.55	0.853
Wearing gloves	1.51	0.54-4.24	0.433	1.53	0.54-4.31	0.422
Salivary cotinine levels	2.24	0.78-6.47	0.135	2.43	0.80-7.38	0.116

Note: [a]= Semen morphology (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 26, based on results from binary logistic regression predicting sperm count among male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2), there were no significant associations between factors with sperm count.

Table 26. Binary logistic regression predicting sperm count[a] among male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2) (n= 62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	1.31	0.38-4.56	0.674	1.31	0.38-4.57	0.674
Smoking	1.00	0.33-2.99	1.000	1.01	0.34-3.03	0.987
Stress	2.33	0.58-9.39	0.233	2.31	0.57-9.41	0.242
Working experience	0.88	0.28-2.78	0.821	0.38	0.04-3.59	0.400
Day spent working	1.25	0.21-7.51	0.807	1.30	0.21-8.29	0.779
Hour spent working	0.95	0.22-4.15	0.941	0.89	0.19-4.05	0.889
Wearing gloves	0.67	0.21-2.08	0.473	0.65	0.20-2.07	0.460
Salivary cotinine levels	0.92	0.29-2.82	0.883	0.95	0.29-3.06	0.929

Note: [a]= Sperm count (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 27, based on results from binary logistic regression predicting semen motility among male tobacco farmers in the end of cultivation season period (T3), there were no significant associations between factors with semen motility.

Table 27. Binary logistic regression predicting semen motility[a] among male tobacco farmers in the end of cultivation season period (T3) (n= 62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	1.76	0.34-9.09	0.502	1.73	0.33-9.01	0.514
Smoking	0.66	0.18-2.36	0.522	0.67	0.19-2.40	0.537
Stress	3.55	0.95-13.20	0.059	3.33	0.89-12.63	0.077
Working experience	0.55	0.09-3.52	0.525	0.55	0.09-3.52	0.525
Day spent working	0.49	0.11-2.56	0.359	0.36	0.05-2.49	0.298
Hour spent working	0.59	0.14-2.47	0.473	0.47	0.11-2.09	0.322
Wearing gloves	1.63	0.43-6.14	0.470	1.59	0.42-6.07	0.495
Salivary cotinine levels	N/A	N/A	N/A	N/A	N/A	N/A

Note: [a]= Semen motility (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 28, based on results from binary logistic regression predicting semen morphology among male tobacco farmers in the end of cultivation season period (T3), there were no significant associations between factors with semen morphology.

Table 28. Binary logistic regression predicting semen morphology[a] among male tobacco farmers in the end of cultivation season period (T3) (n= 62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	0.56	0.14-2.23	0.414	0.56	0.14-2.23	0.414
Smoking	1.00	0.28-3.53	1.000	0.98	0.28-3.48	0.975
Stress	2.26	0.61-8.45	0.225	2.46	0.64-9.49	0.191
Working experience	0.31	0.08-1.12	0.074	0.18	0.03-1.09	0.062
Day spent working	2.42	0.28-21.16	0.426	1.16	0.12-11.73	0.899
Hour spent working	2.41	0.28-21.16	0.426	2.40	0.49-11.79	0.281
Wearing gloves	1.05	0.29-3.78	0.940	1.08	0.29-3.92	0.907
Salivary cotinine levels	N/A	N/A	N/A	N/A	N/A	N/A

Note: [a]= Semen morphology (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

As shown in Table 29, based on results from binary logistic regression predicting sperm count among male tobacco farmers in the end of cultivation season period (T3), hour spent working was significantly reduced sperm count by an average of 73% (OR = 0.27, 95% CI 0.08-0.88, p = 0.029). Moreover, wearing gloves was significantly associated with sperm count (p = 0.021), and wearing gloves increased the odds of sperm count 3.49 times (OR = 3.49, 95% CI 1.20-10.12). Then, after adjusting for age and smoking, we found that hour spent working (OR = 0.27, 95% CI = 0.08-0.94, p = 0.039) and wearing gloves (OR=3.49, 95% CI = 1.20-10.12, p = 0.023) were independently associated with sperm count.

Table 29. Binary logistic regression predicting sperm count[a] among male tobacco farmers in the end of cultivation season period (T3) (n= 62)

Variables	Univariate		p-value	Adjusted**		p-value
	OR	95% CI		OR	95% CI	
Age	0.59	0.18-1.91	0.382	0.58	0.18-1.88	0.364
Smoking	0.67	0.25-1.85	0.443	0.66	0.24-1.82	0.421
Stress	2.35	0.75-7.35	0.141	2.43	0.76-7.77	0.134
Working experience	0.50	0.17-1.44	0.198	0.39	0.07-2.06	0.265
Day spent working	1.19	0.30-4.72	0.805	1.12	0.27-4.62	0.871
Hour spent working	0.27	0.08-0.88	0.029*	0.27	0.08-0.94	0.039*
Wearing gloves	3.49	1.20-10.12	0.021*	3.49	1.20-10.12	0.023*
Salivary cotinine levels	2.72	0.23-31.69	0.424	2.69	0.21-33.78	0.444

Note: [a]= Sperm count (Normal=0, Abnormal=1)
*Association significant at p-value < 0.05, ** Adjusted with age and smoking

CHAPTER V
DISCUSSION

This study is a longitudinal study. The purpose of the study was to examine the association of work-related factors on salivary cotinine levels and semen quality among male tobacco farmers in Sukhothai Province, Thailand. These sections were presented the discussion of findings, conclusion, limitations, recommendations and further study. Moreover, the discussion of findings will be supported by comparing and contrasting with previous relevant studies. This study was conducted from March to May 2022 with sixty-two male tobacco farmers who lived in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province. The questionnaires were used to interview the male tobacco farmers about general characteristics, work-related factors and detailed information about tobacco plantation. Semen quality including volume, pH, viscosity, motility, morphology, and sperm count were analyzed using semen. Salivary samples were analyzed for nicotine exposure levels. Data analysis included descriptive and inferential statistics. Descriptive statistics comprised of frequency and percentage were adopted to analyze the participants' socio-demographic characteristics. Friedman Test was used to compare the salivary cotinine levels between three season periods, while Repeated-measure ANOVA was used to compare mean differences of semen quality at three season periods. Binary logistic regression was used to analyze an association between individual factors, work-related factors with salivary cotinine levels. Multiple linear regression was used to analyze an association between individual factors, work-related factors with semen quality.

There are five sections of this chapter are as follows,

5.1 Discussion of Findings

5.2 Conclusion

5.3 Limitations

5.4 Recommendations

5.5 Further Study

5.1 Discussion of Findings

      5.1.1 Assessment of exposure to nicotine and personal protective behaviors

Based on the study findings of this study, most of tobacco farmers had 2 to 3 times per years of growing seasons (71.0%). 87.1% of male tobacco farmers were owner and do it themselves. The history of male tobacco farmers with start working as an agricultural worker/farmer, most of male tobacco farmers start working were less than 20 years old (77.4%). The major of male tobacco farmers had experience with tobacco plantation more than 20 years (64.5%). In Thailand, the average number of years of work in tobacco farming was 31 years, ranging from 1 to 62 years, and 82.9% worked for 8 h or less per day. They started working when they were young and continued working until they retired or could not work anymore. In Brazil, 63.7% of them worked less than 20 years. They worked very hard and worked from 9 to 18 h per day (Riquinho, 2012).

In addition, most of them did all processing for tobacco plantation (90.3%). The processing for tobacco plantations were 4 processing, the experience with seeding growing, most of male tobacco farmers worked 4-7 days per week (87.1%). 69.4% of male tobacco farmers worked less than 8 hours per day, the experience with transplanting to the tobacco field, most of male tobacco farmers worked 4-7 days per week (79.0%). 51.6% of male tobacco farmers worked more than 8 hours per day, the experience with picking tobacco leaves, most of male tobacco farmers worked 4-7 days per week (90.3%). 83.9% of male tobacco farmers worked less than 8 hours per day, the experience with drying tobacco leaves, most of male tobacco farmers worked 4-7 days per week (83.9%). 66.1% of male tobacco farmers worked less than 8 hours per day. Furthermore, most of the male tobacco farmers reported that they always used PPE (boots and long-sleeved shirt). However, 35.5%, 43.5% and 59.7% of the male tobacco farmers had never worn a raincoat, chemical-resistant rubber gloves and a plastic apron, respectively. Moreover, most of tobacco farmers had shower and clean body immediately after work (95.2%), immediately take a bath and change clothes when finished job (85.5%) and work clothes washed separate with other clothes (77.4%), respectively.

5.1.2 Assessment of salivary cotinine levels and to evaluate the salivary cotinine levels different between the season periods

This study aimed to test the hypothesis that there is difference of salivary cotinine levels between season periods among male tobacco farmers in Sukhothai Province, Thailand. Gas chromatography-nitrogen phosphorous detection (GC) is a valid, reliable, and commonly used quantitative method to measure cotinine in human urine or saliva (Feyerabend, 1990). However, GC is a time-consuming and relatively expensive method. An alternative method that was chosen in the present study was the NicAlert^{TM} saliva strips test (NCTS) because the test can detect as little as 10 ng/mL cotinine. Furthermore, it requires minimal training to use reliably, can be used anywhere, and provides result within approximately 30 minutes only. In general, providing a urine sample is often unacceptable to people and it is rather difficult to arrange in some settings, but collecting saliva specimen is likely to be more acceptable (Peralta, 2001).

The diagnosis accuracy of NCTS when used with saliva was 99% sensitivity and 96% specificity. In this study, it was found that all the testing in three times, in the test of T1, T2 and T3 was found that in T2 have number of saliva cotinine exposure on level 2,3 and 4 more than T1 and T3 that measure by NCTS strip test. Furthermore, the salivary cotinine levels of male tobacco farmers in picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly higher than those of male tobacco farmers in picking first of tobacco plants (T1) and male tobacco farmers in the end of cultivation season period (T3) at p-value < 0.05 (X^2 = 60.18). The result revealed that the salivary cotinine levels are significant different between picking first of tobacco plants (T1), picking top of tobacco plants and dry curing of tobacco plants (T2) and the end of cultivation season period (T3). Dried tobacco was the final process of Thai traditional tobacco cultivation, there was carried out by tobacco farmers through the process. At the harvesting season period, most of the male tobacco farmers worked at farm about four to seven days a week. Moreover, farmers use their hands and arms when working and they might easily contact with sap and gum from tobacco leaves when transferring it into the place where tobacco leaves are cured. Besides that, most of them did not were a raincoat, chemical-resistant rubber

gloves and a plastic apron to protect them from the nicotine exposure. They explained that there were not comfortable when they worked in the farm. Some of them got the symptom such as skin irritated especially when they worked with wet clothe in the early morning. Wet clothing may increase exposure to nicotine from tobacco leaves via dermal absorption because tobacco farmers in this area grown Burley tobacco. The tobacco leaves have different properties than other types. It has a transparent structure, absorbs aromatic, flavored water well. In addition, it has high nicotine concentration, and it has a transparent structure, absorbs aromatic, flavored water well. Previous studies have reported that nicotine level in the farm of tobacco farmers was very high level (Yoo, 2014). Such finding support to the study of Park SJ et al. found that significantly cotinine concentration higher during the harvesting season than during the non-harvesting season (Park, 2018). Furthermore, it was also reported that nicotine was genotoxic at the concentrations found in saliva achieved during tobacco chewing (Trivedi, 1990). In order to be accepted as providing sufficient protection, the suit and gloves should be lightweight and comfortable enough for tobacco farmers who have to work in a hot climate. To reverse these trends, health education program should be recommended to increase knowledge and promote understanding of health adverse effects of tobacco plantations especially to recommendation for personal protection of working in tobacco farms.

5.1.3 Assessment of semen quality and to evaluate the semen quality different between the season periods

Based on the finding of this study, the measure of mean differences at three season periods in the male tobacco farmers showed that sperm parameters (volume, pH, motility, morphology and sperm count) in the picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly lower than those in the picking first of tobacco plants (T1) and those in the end of cultivation season period (T3) at p-value < 0.05. Likewise, the study on the cigarette combustion produces that may have a negative effect on cigarette smoke on the sperm parameters, the effects on the semen of nicotine, substances contained in the tobacco plant. And the main components of cigarette smoke which shows that this alkaloid can change the parameters of sperm (Yoo, 2014). Furthermore, a study on effects of smoking and the

impact on semen parameters found that smokers volunteers have a significant change in the distribution of sperm and cotinine while the negative impact on the motility, morphology and pH of the semen compared to fertility (Condorelli, 2018). This was consistent with Tamer M. Said and colleague's study. They found that using tobacco can reduced sperm quality (Ranganathan, 2019). Furthermore, many studies have reported decrease semen quality, hormonal abnormalities and impaired sperm formation, the growth of sperm, and sperm function in smokers compared with non-smokers (Said, 2005). In this study, the mean of volume, motility, morphology and sperm count of male tobacco farmers who worked in the picking top of tobacco plants and dry curing of tobacco plants (T2) decreased from those male tobacco farmers who worked in the picking first of tobacco plants (T1) and those in the end of cultivation period, with a statistically significant difference between measurement time. This finding supports the effects of nicotine reducing semen quality among male tobacco farmers. It is reasonable to assume that the seasonal of cultivation effect to semen quality. A low-dose nicotine exposure caused major effects on the reproductive potential (Budin, 2017)

5.1.4 The association between individual factors, work-related factors with salivary cotinine levels

In this study, based on results from binary logistic regression predicting salivary cotinine levels in male tobacco farmers at the picking first of tobacco plants (T1), age was significantly associated with salivary cotinine levels (p = 0.045). Age was significantly reduced salivary cotinine levels by an average of 8% (OR = 0.92, 95% CI 0.85-0.99). Moreover, the alcohol intake was significantly associated with salivary cotinine levels (p = 0.011), and alcohol intake was significantly reduced salivary cotinine levels by an average of 77% (OR = 4.43, 95% CI 1.41-13.93). Then, after adjusting for age and smoking, we found that age (OR = 0.92, 95% CI = 0.85-0.99, p = 0.044) and the alcohol intake (OR=5.93, 95% CI = 1.66-21.21, p = 0.006) were independently associated with salivary cotinine levels.

Similarly, a systematic review and meta-analysis showed that age influenced nicotine metabolism, a clearance of nicotine in the elderly was more decreased than adult. It means that nicotine metabolism in the elderly is low (Benowitz, 2009). Moreover,

other studied have found the half-life of urine cotinine was longer in children than to the cotinine half-life in adults (Dempsey, 2013). In addition, Noah R Gubner and colleagues found that chronic alcohol abuse may increase the rate of nicotine metabolism (Gubner, 2016).

While working experience, day and hour spent working increased the odds of salivary cotinine levels. In this study, male tobacco farmers have been worked in the farm more than 20 years and most of them worked at farm about four to seven days a week.

Furthermore, wearing gloves, raincoat and plastic apron reduced the odds of salivary cotinine levels. In this study, most of them did not were a raincoat, chemical-resistant rubber gloves and a plastic apron to protect them from the nicotine exposure. They explained that there were not comfortable when they worked in the farm. Wet clothing may increase exposure to nicotine from tobacco leaves via dermal absorption. The results of efficacy of personal protective equipment for skin against nicotine absorption through the skin route in tobacco workers. The 0.22 mm thick nicotine gloves can use to protect the workers' skin against nicotine exposure (Foddis, 2012). For the tobacco farmers in this study, knowing about PPE was not enough to ensure exposure safety. Therefore, further education regarding PPE is recommended to reduce the risks of nicotine toxicity among tobacco farmers. To promote the safe handling of nicotine, educating the workers is vital to achieve necessary behavioral changes. Health personnel need to educate tobacco farmers about the safe handling of tobacco and keep themselves up to date on current knowledge about nicotine. This is because disseminating information about safety measures through online media is not particularly effective, especially regarding the use of protective raincoat or aprons. A more effective way of educating the workforce is for health workers to explain the pros and cons. Similarly, we should explain the way that they can do it by themselves, a substantial amount of nicotine was transferred to the hands but washing with soap and water in the field significantly reduced nicotine levels by an average of 96% (Curwin, 2005).

However, the result from binary logistic regression predicting salivary cotinine levels of male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco

plants (T2) and those in the end of cultivation season period (T3), there were no significant associations between factors with salivary cotinine levels.

5.1.4 The association between individual factors, work-related factors and salivary cotinine levels with semen quality

Based on results from binary logistic regression predicting semen quality of male tobacco farmers, working experience was significantly positively associated with semen motility in the end of cultivation (T3). In this study, most of male tobacco farmers who had working experience more than 20 years and they worked for all of the day and everyday may be exposed to nicotine for prolong periods of time. Previous study has reported that heavy cigarette smoking (≥20 cigarettes smoked daily) was related to lower semen volume and total sperm count compared with never smoke (Tang, 2019).

Moreover, hour spent working was significantly positively associated with sperm count. These results were like a study of cigarette smokers, which found that effect size was higher in infertile men than in the general population and in moderate/heavy smokers than in mild smokers (Sharma, 2016). H. Asare-Anane and colleagues found that that the number of cigarette sticks smoked per day had no significant effect on semen volume, but significantly reduced semen pH, sperm motility, viability, morphology, and total sperm count (Asare-Anane, 2016). The ability to reverse the damage may depend on the timing of exposure. While eliminating an environmental toxin may help improve semen parameters in men presenting with subfertility, improving semen parameters in men who were exposed to toxic agents in utero may prove more difficult. Small studies suggest that smoking cessation may improve semen parameters (Prentki Santos, 2011).

Furthermore, the association between use of personal protective equipment (PPE) during the dried tobacco production process (T3) with sperm count was also found. In fact, lack of use of PPE is considered one of risk factor of health risk effect. In addition, during the process of picking tobacco leaves and curing tobacco leaves, the farmers use their hands contact the juice and sap of the plants when they transfer the leaves from the agricultural carts to the place of curing in their home or nearby places. The farmers usually use a cloth glove, it does not protect them from exposure

to nicotine on their hands because hot climate may promote sweating on their hands. Similarly, the finding of previous study that a variety of seamless knitted hand gloves were tested to determine prevention of dermal nicotine absorption and nylon gloves were found to be most durable and suitable in all the processes of tobacco cultivation (Gehlbach, 1979) and the use of any type of gloves significantly reduced the levels of nicotine and cotinine in the urine (Doctor, 2004).

While stress reduced the odds of semen quality. Stressful life events may be associated with decreased semen quality. Most investigators have reported an association between higher stress levels and lower semen quality (Hjollund et al., 2004). Stress can affect male fertility through a variety of mechanisms, mainly by changes in testosterone secretion and through disruption of the septum of the testicles blood-testis barrier (Nargund, 2015). Axis inhibition hypothalamic-pituitary-gonadal by the effect of inhibiting hormones gonadotropin-inhibitory (Ubuka, 2014) and stimulation of the hypothalamic-pituitary-adrenal axis by creating an inhibitory effect on the hypothalamic-pituitary-gonadal and Leydig cells, consequently impairs spermatogenesis (Kirby, 2009). Similarly, salivary cotinine levels reduced the odds of semen quality. The fact that nicotine and its water-soluble metabolite cotinine are detectable in the seminal plasma of smokers suggests that other harmful components of tobacco smoke would pass through the blood–testis barrier (Vine, 1993).

In addition, smoking and living with smoker reduced the odds of semen quality. From the article reviewed was shown that smoking duration were negative correlation with the percentage of motility (Hussein, Algadaa, El Faras, & El Fiky, 2011). From the article reviewed was shown that high-dose environmental cigarette smoke, composed of 89 % sidestream cigarette smoke and 11 % the mainstream cigarette smoke, is able to produce oxidative stress and DNA damage in sperm cells. (La Maestra, De Flora, & Micale, 2014) Furthermore, binary logistic regression analysis will be used to adjust the confounding factors of sperm effects.

In this study, according to the data collection of semen samples were conducted for three months. In fact, sperm quality will be changed within 90 days. Therefore, it should be monitored more long term to observe long term changes.

5.2 Conclusion

The mean of sperm volume, pH, motility, morphology and sperm count of male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly lower than those who worked in the picking first of tobacco plants (T1) and those who worked in the end of cultivation season period (T3) while the salivary cotinine levels of male tobacco farmers in the picking top of tobacco plants and dry curing of tobacco plants (T2) were significantly higher than those of male tobacco farmers in the picking first of tobacco plants (T1) and male tobacco farmers in the end of cultivation season period (T3). Age and alcohol intake were significantly associated with salivary cotinine levels. Working experience was significantly positively associated with semen motility. Moreover, hour spent working was significantly positively associated with sperm count. Similarly, wearing gloves was significantly positively associated with sperm count. The study findings confirm that there is difference of semen quality and salivary cotinine levels between season periods. Therefore, education and prevention programs should be directly towards specific risk group. In addition, male tobacco farmers should be advised about the potential adverse effects of their work on sperm quality.

5.3 Limitations

The study also has certain several limitations which and be addressed. One major limitation is that only one research area was selected, although we conducted with male tobacco farmers in the most tobacco plantation area but the sample size was small. It might be the sensitive issue of them. Secondly, the male tobacco farmers had to go to work to collect tobacco leaves in the early morning at the farm and then come back to continuous working at home, this made it difficult to collect sperm during the day and there was a limitation on sperm analysis time as it must be analyzed within one hour after collection.

5.4 Recommendations

Our findings suggested that the male tobacco farmers who fully completed this study might be understand the consequences of nicotine exposure in tobacco farmers and be aware about their health. Furthermore, we therefore recommended that the

health education program should be recommended to increase knowledge and promote understanding of health adverse effects of tobacco plantations especially to recommendation for personal protection of working in tobacco farms.

5.5 Further Study

5.5.1 There should include additional assessments and measurements of nicotine in the environment to know the level of nicotine in the environment that may affect the health of people in the area.

5.5.2 Prospective studies should concern about the time of sperm collection.

5.5.3 Sperm collection should be monitored more long term to observe long term changes.

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