



UNIVERSITI PUTRA MALAYSIA

**CORRELATION OF SACROSCIATIC LIGAMENT RELAXATION AND
SERUM PROGESTERONE CONCENTRATION IN PERIPARTURIENT
SAANEN GOATS**

LOH CHEE HEE

**Ip
FPV 2022 99**

**CORRELATION OF SACROSCIATIC LIGAMENT RELAXATION AND
SERUM PROGESTERONE CONCENTRATION IN PERIPARTURIENT
SAANEN GOATS**

The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. It features a red and white design with a central vertical element and a book at the top. The letters 'UPM' are prominently displayed in a red box at the top left of the shield.

LOH CHEE HEE

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

2022/2023

CERTIFICATION

It is hereby that we have read this project paper entitled “Correlation of Sacrosciatic Ligament Relaxation and Serum Progesterone Concentration in Periparturient Saanen Goat”, by Loh Chee Hee and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

DR. MARK HIEW WEN HAN

DVM (UPM), PhD (Purdue University)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

DR. MOHD MOKRISH MD. AJAT

Bachelor of Science (Hons) (UPM), PhD (Utrecht University)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

ACKNOWLEDGEMENT

The completion of this final year project (FYP) required contributions from a large group of people. As such, I would like to take this opportunity to convey my utmost appreciation to those involved and helped me along this journey. To begin, I would love to express my sincere gratitude to my supervisor, Dr. Mark Hiew Wen Han for his dedication to assist me throughout the journey. His guidance and knowledgeable advice were the most important factors that enable this project to be completed. On top of that, I am deeply thankful to Dr. Mohd Mokrish Md Ajat for giving his thoughtful comments and advice before my presentation. Not only that, Dr. Rasul and Dr. Michelle have been really helpful to me in generating the results for this project, the results were impossible to attain without them.

Besides, I could not have undertaken this journey without the support of my family and friends. Words could not express how thankful I am to everyone who had helped contribute to this project. Thank you.

CONTENTS

	Page
TITLE.....	i
CERTIFICATION.....	ii
ACKNOWLEDGEMENT.....	iv
CONTENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
LIST OF ABBREVIATIONS.....	ix
LIST OF APPENDICES.....	x
ABSTRAK.....	xi
ABSTRACT.....	xiv
1.0 INTRODUCTION.....	1
2.0 LITERATURE REVIEW.....	3
2.1 GOATS.....	3
2.2 GOATS IN MALAYSIA.....	4
2.3 SAANEN GOAT.....	5
2.4 PARTURITION.....	6
2.4.1 SACROSCIATIC LIGAMENT RELAXATION..	8
2.4.2 PROGESTERONE.....	8
2.5 DYSTOCIA IN GOATS.....	9

2.6	ELISA.....	10
3.0	MATERIALS AND METHOD.....	12
3.1	ANIMAL.....	12
3.2	SACROSCIATIC LIGAMENT RELAXATION.....	14
3.3	BLOOD COLLECTION.....	15
3.4	QUANTITATIVE MEASUREMENT OF SERUM.....	16
	PROGESTERONE USING ELISA TEST KIT.....	
3.5	STATISTICAL ANALYSIS.....	17
4.0	RESULTS.....	20
4.1	DESCRIPTIVE STATISTICS.....	20
4.2	TEST OF NORMALITY.....	23
4.3	CORRELATION ANALYSIS.....	25
5.0	DISCUSSION.....	29
5.1	PROGESTERONE.....	29
5.2	SACROSCIATIC LIGAMENT RELAXATION.....	30
6.0	CONCLUSION.....	32
7.0	RECOMMENDATION.....	32
	REFERENCES.....	33

LIST OF TABLES

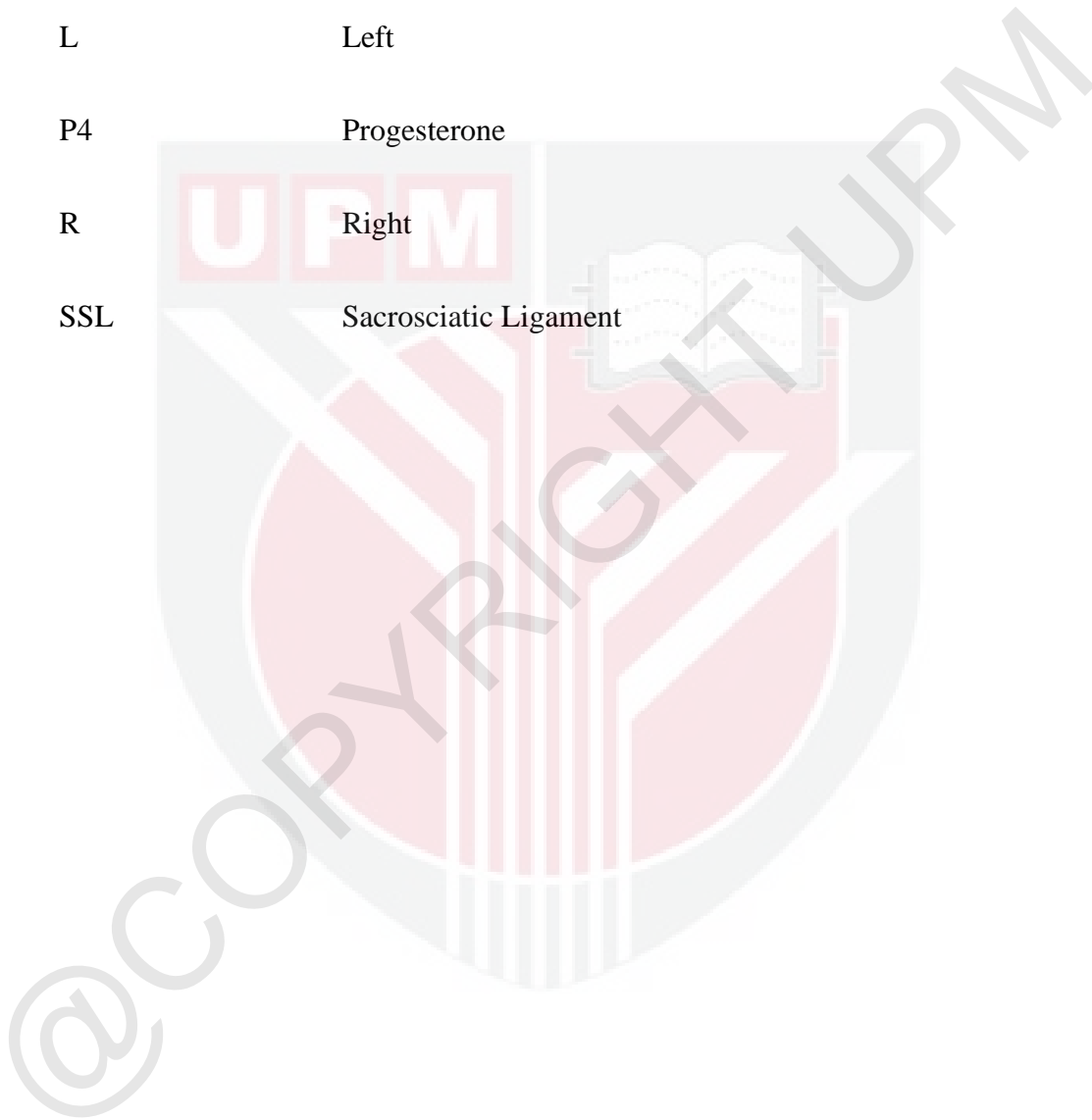
	Page
Table 1. Progesterone concentration of 6 periparturient Saanen goats	19
Table 2. Left sacrosciatic ligament relaxation of 6 periparturient Saanen goats	20
Table 3. Right sacrosciatic ligament relaxation of 6 periparturient Saanen goats	21
Table 4. Normality tests of progesterone according to parturition day of 6 periparturient Saanen goats	23
Table 5. Normality tests of left and right SSL according to parturition day of 6 periparturient Saanen goats	24
Table 6. Pearson's correlation analysis of P4 concentration and parturition day of 6 periparturient Saanen goats	25
Table 7. Pearson's correlation analysis of left and right SSL and parturition day of 6 periparturient Saanen goats	26
Table 8. Pearson's correlation analysis of P4 concentration, left and right SSL and parturition day of 6 periparturient Saanen goats	27

LIST OF FIGURES

	Page
Figure 1. Saanen does	12
Figure 2. ITAFoS Signboard	13
Figure 3. Sacrosciatic ligament relaxation measurement	14
Figure 4. Jugular venipuncture	15
Figure 5. Cryovials with serum stored at -20°C	15
Figure 6. FR E-2500 LDN, Germany	17
Figure 7. Tecan Sunrise microplate reader	17
Figure 8. Serum progesterone concentration for 6 Saanen does in relation to the day of parturition	19
Figure 9. Left and right SSL relaxation to parturition day of 6 periparturient Saanen goats	21

LIST OF ABBREVIATIONS

L	Left
P4	Progesterone
R	Right
SSL	Sacrosciatic Ligament



LIST OF APPENDICES

No.	Title	Page
Appendix A:	List of statistical analyses.....	39
1.	Independent samples t-test of P4..... concentration between Day -1 to Day 0..	39
2.	Independent sample t-test of P4..... concentration between Day 0 to Day 1...	40
3.	Independent samples t-test of P4..... concentration between Day 1 to Day 2...	41
4.	Independent samples t-test of P4..... concentration between Day 2 to Day 3...	42
5.	Independent samples t-test of left and.... right SSL between Day -1 to Day 0.....	43
6.	Independent samples t-test of left and.... right SSL between Day 0 to Day 1.....	44
7.	Independent samples t-test of left and.... right SSL between Day 1 to Day 2.....	45
8.	Independent samples t-test of left and.... right SSL between Day 2 and Day 3.....	46

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Project Tahun Akhir.

**KORELASI LIGAMEN SACROSCIATIC DAN KEPEKATAN
PROGESTERON SERUM DALAM KAMBING SAANEN SEKITAR MASA
BERANAK**

Oleh

Loh Chee Hee

2022

Penyelia: Dr Mark Hiew Wen Han

Penyelia bersama: Dr Mohd Mokrish Md. Ajat

Penternakan kambing telah berkembang dengan kadar yang agak perlahan berbanding spesies ruminan lain di sekeliling dunia. Kadar produktiviti yang rendah dan isu pembiakan telah menjadikannya tidak menguntungkan kepada banyak perniagaan. Distokia adalah isu pembiakan yang biasa berlaku pada kambing di seluruh dunia dengan kelaziman sehingga 50%, tertinggi di kalangan spesies ruminan. Kira-kira 50% daripada populasi kambing tinggal di negara-negara yang sedang membangun dan majoriti penternak kambing tidak memelihara kambing secara komersil. Oleh itu, masa mengawan kambing biasanya tidak direkodkan dengan tepat. Selain itu, kerugian ekonomi yang ditanggung disebabkan kambing boleh menjadi beban besar kepada penternak. Oleh itu, bantuan sekitar masa beranak amat penting untuk mengurangkan komplikasi kepada ibu dan anak kambing untuk mengurangkan kerugian. Sekitar masa beranak, kambing akan menunjukkan tanda-tanda yang membolehkan penternak meramalkan masa beranak. Akan tetapi, kebanyakan petunjuk adalah subjektif. Matlamat kajian ini adalah untuk menentukan kebolehlaksanaan penggunaan kelonggaran ligament sacrosciatic (SSL) dan kepekatan hormone progesterone (P4) di dalam serum untuk mengesan masa beranak kambing. Sebanyak 6 ekor kambing Saanen telah dikaji. Kelonggaran SSL dan kepekatan P4 setiap betina diukur dari satu hari sebelum bersalin hingga 3 hari selepas bersalin. Kelonggaran SSL diukur menggunakan 2 pembaris logam yang diletakkan berserenjang antara satu sama lain di antara sacrum dan tuber ischii, manakala kepekatan P4 dianalisis menggunakan ELISA. Keputusan menunjukkan bahawa korelasi antara hari sekitar masa beranak dan kepekatan P4 adalah signifikan ($P < 0.05$). Tetapi, korelasi antara kelonggaran SSL dan kepekatan P4 tidak signifikan. Kesimpulannya, kajian ini menunjukkan bahawa kepekatan P4 dapat meramalkan

masa bersalin dalam masa 24 jam selepas permulaan ujian. Kelonggaran SSL dalam semua ibu kambing menunjuk kenaikan sekitar masa beranak, tetapi ianya tidak signifikan.

Kata kunci: *progesterone; kelonggaran ligament sacrosciatic; beranak; distokia; kambing*



ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

**CORRELATION OF SACROSCIATIC LIGAMENT RELAXATION AND
SERUM PROGESTERON CONCENTRATION IN PERIPARTURIENT
SAANEN GOAT**

by

Loh Chee Hee

2022

Supervisor: Dr Mark Hiew Wen Han

Co-supervisor: Dr Mohd Mokrish Md. Ajat

Goat farming has been growing at a relatively slow rate compared to other ruminant species around the globe. Low productive efficacy and reproductive issues have made it an unfavourable to many businesses. Dystocia is a fairly common reproductive issue that occurs in goats worldwide with a prevalence of up to 50%, the highest among all ruminant species. About 50% of the world's goat population reside in developing countries and majority of the goat farmers do not rear goats commercially. Therefore, the time of mating is usually not properly recorded. Besides, economic losses incurred from the goats can be great burden to the farmers. Therefore, it is crucial to provide human intervention to mitigate periparturient complications to both doe and kid to reduce losses. Periparturient goats will show many indications that allow farmers to predict time of parturition, but, most indicators are subjective. The aim of this study was to determine the feasibility of using sacrosciatic ligament relaxation (SSL) and serum progesterone concentration (P4) to detect the time of parturition in late gestation does. A total of 6 Saanen does were employed in the study. SSL relaxation and P4 concentration of each doe were measured from Day 1 prepartum to Day 3 postpartum. SSL relaxation was measured using 2 metal rulers placed perpendicularly to each other between the sacrum and tuber ischii while P4 concentration was analysed using ELISA. Results showed that the correlation between days to parturition and P4 concentration was significant ($p < 0.05$). However, the correlation between SSL relaxation and P4 concentration was not significant. In conclusion, this study demonstrated that P4 concentration is effective in predicting the time of parturition within 24 hours after the onset of testing. While SSL relaxation in all does showed general increment near term, the results were not significant.

Keywords: *progesterone; sacrosciatic ligament relaxation; parturition; dystocia; goat*



1.0 INTRODUCTION

Progesterone is an essential hormone secreted by corpus luteum to maintain pregnancy. In pregnant goats, the serum progesterone level remains within 4.1 ± 0.33 , 5.0 ± 0.21 , 7.1 ± 0.49 , 7.5 ± 0.69 , and 2.05 ± 0.31 ng/mL in the first 5 months of pregnancy (S.O. Hussain, 2015). The progesterone level in serum will reduce significantly approximately 24 – 30h prior to parturition to $< 1.0 - 2.8$ ng/mL (Singer et al., 2004). Laboratory serum progesterone assays are the gold standard in measuring serum progesterone levels. However, they commonly require 24 hours or more to obtain results and are costly.

Relaxation of sacrosciatic ligament has been seen in many animals during parturition to ease delivery process. In cattle, the probability of calving within 24h was accurate with 93.9% parturition observed (31/33) when sacrosciatic ligament measurement increased by ≥ 5 mm (K.D.Shah et al., 2005). However, there is no available study pertaining to sacrosciatic ligament in goat during parturition.

There are many indications of peri-parturient goats. But an accurate and reliable method of predicting parturition in goat that is quick, cost-effective, sensitive and feasible on-site is still underway. Using an accurate, fast and cost-effective method to determine the parturition time of goat will be of farmer's interest to ensure higher production. This study will determine the correlation between the changes in serum progesterone concentration and sacrosciatic ligament relaxation with the time of parturition. Therefore, the objectives of this study are to:

1. Determine the correlation between sacrosciatic ligament relaxation with serum progesterone concentration at the time of parturition in Saanen goats.

2. Determine the feasibility of using sacrosciatic ligament relaxation as a method to predict parturition.

The hypotheses for this study are:

1. H_0 : There is no correlation between sacrosciatic ligament relaxation and serum progesterone concentration in periparturient Saanen goats
2. H_a : There is correlation between sacrosciatic ligament relaxation and serum progesterone concentration in periparturient Saanen goats.

2.0 LITERATURE REVIEW

2.1 Goats

The goat was the first animal to be domesticated by mankind during the first known civilization, Mesopotamia (Boyazoglu et al., 2005; Zeder & Hesse, 2000). The wild goat, *Capra aegregus*, was domesticated and is now known as *Capra hircus* under the Bovidae family. Goats are found all over the world and are adaptable to dry and humid conditions, as well as tropical and temperate climates. In many parts of the world, they are valued for a wide range of goods and uses like meat, milk, cheese, yogurt, mohair, cashmere, companion, and recreation (Devendra & Haenlein, 2016).

Different breeds of goats serve different purposes based on their distinctive characteristics such as Boer for meat, Saanen for milk and various dairy products, and Angora for cashmere (Devendra & Haenlein, 2016). Most commercial goat breeds nowadays have been selectively bred for centuries to improve and increase specific desired characteristics.

It is estimated that out of a total of 570 goat breeds, only 69 are single-purpose dairy types. Within that, 36 breeds (about 52%) originated from Europe, followed by 25 breeds (37%) in Asia and 8 breeds (11%) in Africa. In developing countries, the majority of goat breeds are dual-purpose breeds used mainly for meat production and medium to low yield milk production (Devendra & Haenlein, 2016).

Nearly 60% of the world's goat population are found in Asia with China, India, Pakistan and Bangladesh having the highest population (Miller & Lu, 2019). India is the greatest goat milk producer taking up 22% of international goat milk trade. According to Dubeuf et al. (2004), Australia is the most important goat meat exporter taking up 65% of the trade. South Africa is the major mohair producer producing up

to 50% of the mohair in the world. China is the principal cashmere exporter having influence over 65% of the market.

For centuries, humans have used goats for many purposes. However, the goat sector still remains an unfavorable business in many countries because of its relatively low productive efficiency compared to other livestock (Dubeuf et al., 2004).

2.2 Goats in Malaysia

As of 2021, there is an estimated 328,944 heads of goats in Malaysia with more than 85% of the population residing in Peninsular Malaysia (Department of Veterinary Services, 2021). In Malaysia, goats serve 2 main purposes, providing mainly meat to the local market with medium to low milk production on the side (Devendra & Haenlein, 2016). Malaysia has only 1 indigenous goat of its own, known as Katjang, a meat-producing goat (Khandoker et al., 2017). Other exotic goat breeds such as Boer, Jamnapari and Savanna have been imported to Malaysia for many years to serve various markets (meat and dairy) and for crossbreeding purpose (Khandoker et al., 2017; Devendra & Haenlein, 2016; Abu, 2014).

The meat of domestic goat (*Capra hircus*) is known as chevon (adult goat) and cabrito (young goat). While the meat of domestic sheep (*Ovis aries*) is called mutton (adult sheep) and lamb (young sheep). However, mutton is commonly used in Malaysia as the term for both goat and lamb meat (Kaur, 2010). The self-sufficiency level of mutton (comprised of goat and sheep) in Malaysia was 11.45% in 2015 and has been steadily decreasing ever since. In 2020, the self-sufficiency level was only 8.21% and depended highly on importation (Department of Veterinary Services, 2020). The consumption of mutton was 1.3kg per capita in 2021, showing extremely low

consumption that could be due to general disinterest in mutton consumption in Malaysia, shortage of mutton supply or relatively high price of mutton (RM38/kg) compared to other protein sources (Department of Veterinary Services, 2021).

In dairy goat, despite its low productive efficiency, the establishment of new dairy goat farms is gradually increasing due to increased demand for dairy goat products and better ex-farm price compared to other dairy livestock due to traditional beliefs of its health benefits. Goat milk fetches an ex-farm price of RM20/litre compared to cow milk which is priced at RM2.20/litre. However, most small-scale dairy goat farms are not managed well due to poor management knowledge (Shahudin et al., 2018). Besides the constant challenge of low performance, dairy goat farmers in Malaysia are also facing problems such as lack of local environment adaptation, lack of quality feeds leading to low feed utilization and many tropical diseases (Liang & Paengkoum, 2019; Shahudin et al. 2018). The self-sufficiency level of milk in Malaysia (comprised of cow, buffalo, goat and sheep) was 62.14% and milk consumption per capita (comprised of cow, buffalo, goat and sheep) was 1.9L per capita in 2021 (Department of Veterinary Services, 2021). However, there is no data available for goat milk self-sufficiency ratio and goat milk consumption per capita.

2.3 Saanen Goat

The Saanen goat derived its name from the Saanen valley of central Switzerland, where they originated. The breed has completely white and short haircoat with occasional black spots on the udder, ears, and nose (Encyclopaedia Britannica, 2018). The ears are generally erect and point forward. It has a straight facial line and saber shaped horns that point backward. The average weight of an adult male is 90kg

and female is 65kg. The average height at withers is 90cm for adult males and 80cm for females (Devendra & Haenlein, 2016).

Saanen goat is regarded as the world's best-developed and highest milk producing breed that has been extensively exported around the globe. It is one of the most popular dairy goat breeds in many countries. They produce high milk volume with relatively low levels of milk fat content and is more easily digestible than cow's milk (Encyclopaedia Britannica, 2018; Devendra & Haenlein, 2016).

Many countries have been cross-breeding Saanen selectively with indigenous goat breeds and established specific characteristics that are now distinctively deviated from the original Saanen, namely French Saanen, British Saanen, Dutch White Goat, Bulgarian White, and Banat White to cater to the climate, management, and nutritional availability of different countries (Devendra & Haenlein, 2016). Depending on the nation and management, the annual milk yield can range from 300 to 2000kg per lactation. (Devendra & Haenlein, 2016). Generally, Saanen goats raised in colder weather will have significant increment in milk yield per lactation (Ishag et al., 2012).

2.4 Parturition

Parturition is a process of delivery of the fully grown fetus upon the completion of a normal gestation period. Reproductive activity of goat begins when the females reach puberty, which happens at around 5 to 15 months of age depending on climate, presence of male and nutrition (Sánchez-Dávila et al., 2018). The goat is a seasonal polyestrous mammal, which means it has multiple estrus cycles during the breeding season and is sexually active and receptive to mating. The breeding activities peak

during autumn. The normal gestation period is approximately 150 days (5 months), so a female goat could normally have 2 deliveries per year.

The parturition process in most mammals is categorized into 3 stages. In goat, the first stage of labour lasts around 6 – 12 hours, the second stage around 0.5 – 1 hour and third stage will occur approximately 2 – 12 hours later (Mehta et al., 1990; Jackson, 1995).

Parturition behaviors start to exhibit a few days to several hours prior to parturition. Most does (76%) isolate themselves from the herd a few hours before parturition and the majority of the births (65%) occur during mid-day, between 11 am to 4 pm because this is the period of least activity where does typically lie down and ruminate (Lickliter, 1985). Parturition at midnight is rare (Bosc et al., 1988). Increased restlessness, pacing, pawing the ground, frequent change of position, increased vocalization (short grunts, low-pitched bleats) and increased aggressiveness have been observed. Immediately after birth, does vigorously lick and nudge their kids starting from the head region and slowly progress posteriorly to the entire body for 2 to 4 hours. Dams and kids begin a series of vocal interchanges within minutes of the kid's expulsion (Lickliter, 1985).

Aside from parturition behaviours, physical appearance of periparturient goats is also different from normal non-pregnant goats. Goats nearing parturition will show signs such as raised tail, enlarged udder, relaxed pelvic ligament and swollen vulva (Lickliter, 1985).

2.4.1 Sacrosciatic Ligament Relaxation

Sacrosciatic ligament is a sheath of broad ligament located at the pelvic region from the sacrum to tuber ischii. It is a ligament commonly present in herbivores (Minnesota Veterinary Anatomy, 2021). Estrogen surge at term coupled with increased relaxin causes the relaxation of sacrosciatic ligament to ease the delivery process. In cattle, the probability of estimating calving within 24h was 93.90% accurate (n = 37) when sacrosciatic ligament increased by ≥ 5 mm (Shah et al., 2006). However, there is no available study pertaining to sacrosciatic ligament in goats during the periparturient period.

2.4.2 Progesterone

Various biochemical changes such as hormone levels and metabolic rate will fluctuate during the course of pregnancy. Several days before, during and after parturition, the changes can be drastic. Progesterone is an essential hormone secreted by the corpus luteum to maintain pregnancy in animals. In single fetus-bearing does, the average serum progesterone level falls within 4.8 ± 0.70 , 5.85 ± 0.88 , 8.95 ± 0.72 , 9.15 ± 0.33 , and 4.37 ± 0.62 ng/mL in the 1st, 2nd, 3rd, 4th, and 5th months of pregnancy respectively (Hussain, 2016). It is also stated by Hussain (2016) and Thorburn et al., (1972) that does bearing twin or triplets will have higher serum progesterone concentrations during the gestation period.

During estrus, the progesterone level will be extremely low, at only 0.2 ng/mL (Thorburn et al., 1972). After mating during estrus, serum or fecal progesterone analysis can be done on Day 21 post-breeding to detect pregnancy (Hussain, 2016).

Goats that are not pregnant will have low progesterone due to the regression of corpus luteum, while pregnant goats will have higher than normal progesterone level.

Progesterone level will decline significantly several days prior to parturition. A study found that the average progesterone concentration 0 – 9 h prepartum was 1.48 ± 0.69 ng/mL (Singer et al., 2014). The rapid withdrawal of progesterone in circulation prompts the estrogen production to increase. However, the mechanisms that suppress progesterone near term are still uncertain (Zakar et al., 2007).

Progesterone profile is one of the many parameters farmers use for pregnancy diagnosis in goats. Assessment of progesterone levels during different stages of gestation can be very useful to evaluate the fertility status of the doe, whether any intervention is needed during parturition, and management system (Khanum et al., 2007). Laboratory radioimmunoassay remains the most accurate test to determine progesterone concentration with sensitivity of 0.03 ng/mL, however it must be done in the lab and requires a longer time (Singer et al., 2004). There are also various enzyme immunoassays or ELISA test kits in the market specifically designed to detect serum or plasma progesterone with various sensitivity. These ELISA test kits are able to give quick results with relatively low price and it can be performed in the field.

2.5 Dystocia in goats

Dystocia is a term for abnormal or difficult birthing process. Most published studies generalized the prevalence of dystocia in small ruminant as a whole rather than targeting goats alone. However the incidence of dystocia is considered higher in goats compared to sheep (Sharma et al., 1999; Mehta et al., 2002). Incidence of dystocia in goats varies between 8% to 50%, the highest among ruminants and is caused by a

myriad of factors that are categorized into maternal and fetal causes (Purohit, 2006). Fetal causes include faulty disposition and oversized fetus while maternal causes include incomplete cervical dilatation, uterine torsion, uterine inertia, narrow pelvis, age, parity, length of gestation, maternal health (Purohit, 2006; Sharma, 2015; Majeed et al., 1989).

Dystocia in goats can result in huge economic losses to farmers either due to death of newborn, dam or both and causing adverse effects to future dam's fertility (Mcsporrán, 1980). Human interventions can be done in case of dystocia in goats. Obstetrical mutation and traction and Caesarean section can be performed in viable fetus (Bhattacharyya et al., 2015). While for dead fetus, subcutaneous fetotomy can be performed. However, obstetrical procedures and drugs used during the process will be expensive and not economical especially to smallholders.

2.6 ELISA

Enzyme-linked immunosorbent assay (ELISA), also known as enzyme immunoassay (EIA) is an immunoassay that employs enzymes to detect and quantify substances such as antibodies, antigens, hormones, and cytokines and all sorts of proteins in liquid biological samples. It is a powerful method to detect and quantify a specific protein in a complex mixture via highly specific antibody-antigen interactions (Crowther, 2009).

There are 4 basic types of ELISA namely direct, indirect, sandwich and competitive ELISA but the principle of ELISA tests are similar. The antigen is immobilized on a microplate and is complexed with an antibody that is bound to a reporter enzyme. Detection is accomplished by measuring the activity of the reporter

enzyme via incubation with the appropriate substrate to produce a measurable result which could either be a colour change or optical density difference in relation to the amount of antibodies or antigens in the sample (Crowther, 2009).

The data obtained from ELISA tests can be quantitative, qualitative, or semi-quantitative. For quantitative measurement, the concentrations are plotted and compared to a standard curve. As for qualitative measurement, the results simply confirm or dismiss the presence of a particular protein (usually antigen or antibody) in a sample. The semi-qualitative measurement compares the intensity of signals which will allow the relative quantification of the specific protein level in the samples (Alhajj, 2022).

ELISA tests have become the economic and efficient alternative to many laboratory-bound serodiagnostic tests and still have a relatively high sensitivity and specificity (Crowther, 2009). Many ready-to-use ELISA kits have been developed over the years and are commercially available for detection of hundreds of thousands of specific proteins. The worldwide demand is still exceedingly large (Lequin, 2005).

3.0 MATERIALS AND METHODS

3.1 Animal

This study was approved by the IACUC (International Animal Care and Use Committee) with the reference number: AUP-R07/2019. Six late gestating Saanen does aged between 4 to 8-years-old that were expected to kid within 3 days were used in this study. Data was collected from 3 days before parturition until 3 days after parturition between 8 am and 10am daily before feeding. The does were kept in individual pens at Institute of Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia (UPM) and were fed with goat pellets, Napier grass and *ad libitum* water. These animals were previously estrus synchronized and natural mating was done.



Figure 1. Saanen does



Figure 2. ITAFoS Signboard

3.2 Sacrosciatic Ligament Relaxation

The hair at the rump of the goats at the area of sacrum to tuber ischii was clipped. The sacrosciatic ligament relaxation was measured using two rulers that were placed perpendicularly to each other at the region of the ligament. One ruler was placed parallel to the sacrosciatic ligament which was between sacrum and tuber ischii while another ruler was placed perpendicularly to the first ruler with the bottom edge touching the front edge of the first ruler. The relaxation was measured in the second ruler from the touching point of ligaments to the touching point of the first ruler. The measurement of sacrosciatic ligament relaxation was done daily during the last week of gestation.



Figure 3. Sacrosciatic ligament relaxation measurement

3.3 Blood Collection

Each goat was restrained in a standing position with the head elevated and turned to one side to expose the jugular vein. The jugular vein was then occluded by applying pressure at the base of the jugular groove to visualize the vein. The insertion area was swabbed with alcohol. With the bevel facing up, a 21G needle with 5 mL syringe was inserted through the skin and into the vein at approximately 20-30° angle. A total of 5 mL of blood was collected into plain tubes from each goat. The needle was removed once the collection was completed. Pressure applied at the base of the jugular groove was then released and reapplied at the injection site for 30 to 60 seconds to prevent formation of hematoma. Blood collection was done daily during the last week of gestation until 3 days postpartum. Within 3 hours of collection, the rest of the

blood was spun down at 1500 rpm for 15 minutes and the serum was transferred to cryovial tubes before being stored at -20°C.



Figure 4. Jugular venipuncture

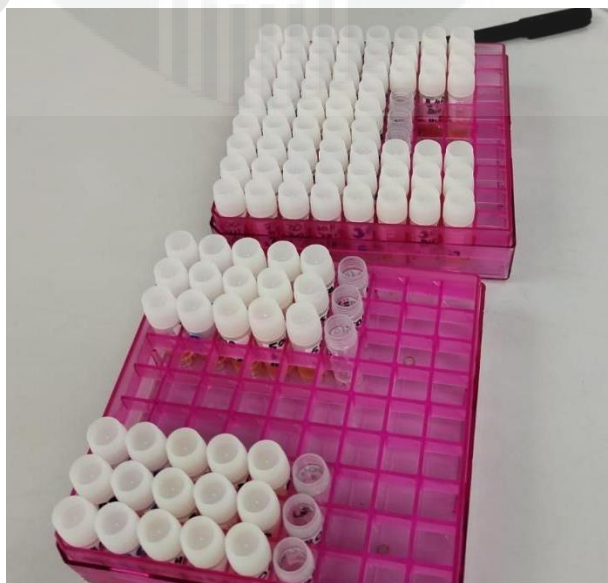


Figure 5. Cryovials with serum stored at -20°C

3.4 Quantitative measurement of serum progesterone using ELISA test kit

Progesterone from the goat serum samples was measured via ELISA (FR E-2500 LDN, Germany). The frozen serum samples were thawed and solutions for the test which consisted of conjugate, wash, substrate and stop solution were brought to room temperature. 25 μ L of Standard A, B, C, D, E, F and G were dispensed into the first 2 columns of the microplate wells (A1, A2: 0 ng/mL; B1, B2: 0.3 ng/mL; C1, C2: 1.25ng/mL; D1, D2: 2.5ng/mL; E1, E2: 5 ng/mL; F1, F2: 15 ng/mL and G1, G2: 40 ng/mL). H1 and H2 were set as negative control. 25 μ L of serum were pipetted into the wells and incubated for 5 minutes at room temperature. Then, 200 μ L of conjugate solution was added into each well and mixed thoroughly for 10 seconds. The mixture was incubated for 60 minutes at room temperature. After the incubation, the wells were rinsed manually 3 times with 300 μ L diluted wash solution and struck sharply on absorbent paper to remove residual droplets. Then, 200 μ L of substrate solution was added to each well and incubated for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 μ L of stop solution in each well and optical density of solution in each well was measured at 450 nm with Tecan | Sunrise™ microplate reader.



Figure 6. FR E-2500 LDN, Germany

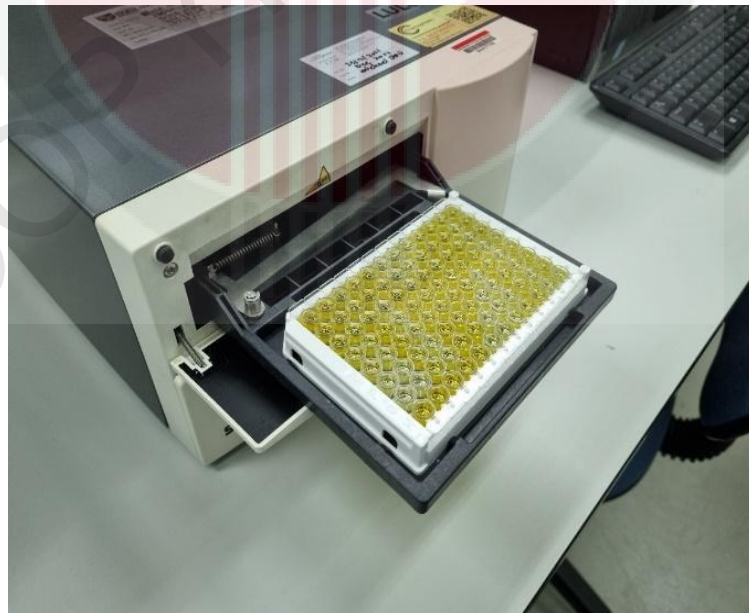


Figure 7. Tecan | Sunrise microplate reader

3.5 Statistical analysis

Data collected were arranged into a spreadsheet (Microsoft Excel 365), reviewed for any missing values or errors and analyzed using IBM SPSS Statistics 23 with P values < 0.05 considered to be significant. The measurement of sacrosciatic ligament relaxation and serum progesterone level were analyzed using the basic descriptive statistics for their mean, standard deviation and ranges. The normality test was done to determine the distribution of data. Independent t-test was done to determine the significant difference between serum progesterone level and parturition day, sacrosciatic ligament relaxation and parturition day as well as serum progesterone level and sacrosciatic ligament relaxation. The correlations between serum progesterone level, sacrosciatic ligament and parturition day were analyzed with Pearson's correlation analysis.

4.0 RESULTS

4.1 Descriptive statistics

Day	Mean P4 (ng/mL)	Standard deviation (ng/mL)
-1	3.770	1.817
0	0.942	0.346
1	0.365	0.222
2	0.287	0.136
3	0.226	0.068

Table 1. Progesterone concentration of 6 periparturient Saanen goats

In Table 1, the average P4 concentration of Saanen goats (n=6) showed a decreasing trend from 3.770 ng/mL on Day -1 to 0.226 ng/mL on Day 3 post-partum. Standard deviation was the highest on Day -1 with 3.770 ± 1.817 ng/mL and lowest on Day 3 post-partum with 0.226 ± 0.068 ng/mL.

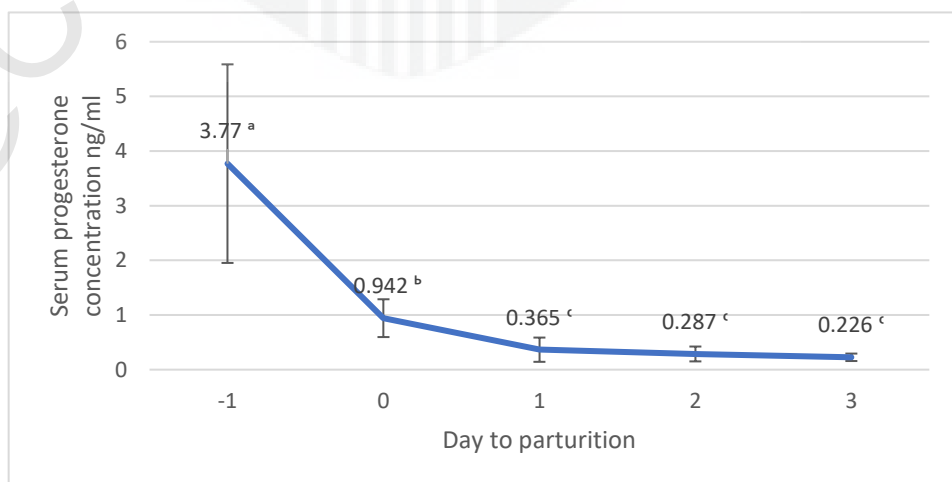


Figure 8. Serum progesterone concentration for 6 Saanen does in relation to the day of parturition

In Figure 1, the P4 concentration showed a significant drop from Day -1 to Day 0 from 3.77 ± 1.817 ng/ml to 0.942 ± 0.346 ng/ml. The P4 concentration continued to decrease 3 days post-partum at a relatively slower rate reaching 0.226 ± 0.068 ng/ml on Day 3 post-partum. The standard deviation 1 day prior to parturition was large and gradually decreased after parturition. The standard deviation of P4 concentration on Day 3 post-partum was the smallest 0.226 ± 0.068 ng/ml. Overall, the P4 concentration showed a decreasing trend before and after parturition.

Day	Mean Left SSL (mm)	Standard deviation (mm)
-1	5.333	3.777
0	7.333	3.204
1	5.333	2.503
2	5.500	2.345
3	4.333	2.503

Table 2. Left sacrosiatic ligament relaxation of 6 periparturient Saanen goats

In Table 2, the average left sacrosiatic ligament relaxation of Saanen goats (n=6) was 5.333 mm on Day -1 and 4.333 mm on Day 3 post-partum, showing a decreasing trend. The peak relaxation was observed on Day 0, at 7.333 ± 3.204 mm. The standard deviation of left sacrosiatic ligament relaxation on all 5 days were large with the highest reaching 5.333 ± 3.777 mm.

Day	Mean Right SSL (mm)	Standard deviation (mm)
-1	6.167	2.137
0	8.167	2.639
1	6.167	0.983
2	6.500	1.378
3	6.667	2.338

Table 3. Right sacrosciatic ligament relaxation of 6 periparturient Saanen goats

In Table 3, the average right sacrosciatic ligament relaxation of Saanen goat (n=6) was 6.167 mm on Day -1 and 6.667 mm on Day 3 post-partum. The peak relaxation was observed on Day 0, which is the parturition day, at 8.167 ± 2.639 mm. The standard deviations of right sacrosciatic ligament relaxation on all 5 days were between 0.9 to 2.6 mm.

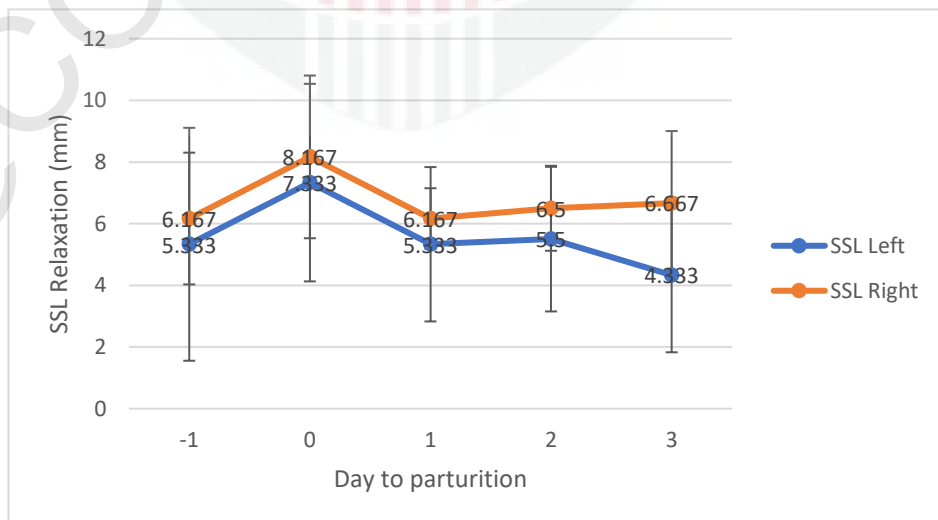


Figure 9. Left and right SSL relaxation to parturition day of 6 periparturient Saanen goats

In Figure 2, the left SSL showed a 60% (2 mm) increment from 5.333 ± 3.777 mm on Day -1 to 7.333 ± 3.204 mm on Day 0. The relaxation dropped back to 5.333 ± 2.503 mm on Day 1 post-partum and reducing to 4.333 ± 2.503 mm on Day 3 post-partum. Similarly, right SSL also showed a 60% (2 mm) increment from 6.167 ± 2.137 mm on Day -1 to 8.167 ± 2.639 mm on Day 0. The relaxation reduced back to 6.167 ± 0.983 mm on Day 1 post-partum and showed a slight increase on both Day 2 and Day 3 post-partum. Overall, both left and right SSL showed a fairly similar trend on – Day 1, Day 0 and Day 1 post-partum. However, the relaxation of right SSL remained slightly elevated at 6.667 ± 2.338 mm on Day 3 post-partum while left SSL decreased to 4.333 ± 2.503 mm.

4.2 Test of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
P401	.247	6	.200*	.905	6	.404
P400	.175	6	.200*	.895	6	.345
P41	.369	6	.011	.751	6	.020
P42	.265	6	.200*	.916	6	.479
P43	.228	6	.200*	.928	6	.563

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Table 4. Normality tests of progesterone according to parturition day of 6 periparturient Saanen goats

Table 4 showed the normality of P4 concentration according to parturition day with P401 indicating P4 concentration on Day -1 and P43 indicating P4 concentration on Day 3 post-partum.

Kolmogorov-Smirnov and Shapiro-Wilk tests were ran and the data followed a normal distribution. Both tests showed $P > 0.05$ for all data, thus data is assumed to be normally distributed. However, Shapiro-Wilk test is more suitable because of the small sample size of this study ($n=6$). Since the data followed a normal distribution, parametric test (Independent t- test) is indicated to compare the groups.

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
SSLL01	.202	6	.200*	.880	6	.268
SSLR01	.318	6	.057	.824	6	.096
SSLL00	.208	6	.200*	.908	6	.425
SSLR00	.209	6	.200*	.847	6	.148
SSLL1	.228	6	.200*	.847	6	.148
SSLR1	.401	6	.003	.770	6	.031
SSLL2	.261	6	.200*	.739	6	.015
SSLR2	.195	6	.200*	.861	6	.191
SSLL3	.228	6	.200*	.847	6	.148
SSLR3	.279	6	.159	.908	6	.421

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Table 5. Normality tests of left and right SSL according to parturition day of 6 periparturient Saanen goats

Table 5 showed the normality of sacrosciatic ligament relaxation of left and right sides according to parturition day. SSLL01 indicating sacrosciatic ligament left on – Day 1 and SSLL3 indicating sacrosciatic ligament left on Day 3 post-partum. Relaxation of SSL right followed the same abbreviation.

Kolmogorov-Smirnov and Shapiro-Wilk tests were ran and the data followed a normal distribution. From Shapiro-Wilk test, majority of the data had $P > 0.05$ except for SSLR1 and SSSL2. However, the data set is still assumed to be normally distributed as majority of the data showed $P > 0.05$ and parametric test (Independent t-test) is indicated to compare the groups.

4.3 Correlation analysis

		Day	P4
Day	Pearson Correlation	1	-.706**
	Sig. (2-tailed)		<.001
	N	30	30
P4	Pearson Correlation	-.706**	1
	Sig. (2-tailed)	<.001	
	N	30	30

** . Correlation is significant at the 0.01 level (2-tailed).

Table 6. Pearson's correlation analysis of P4 concentration and parturition day of 6 periparturient Saanen goats

Table 6 showed the parturition day and P4 concentration were significantly correlated with $P < 0.01$ using Pearson's correlation analysis (parametric test)

because the data were normally distributed. The R value was 0.706, indicating the strong strength of association between P4 concentration and parturition day.

		Day	SSLR	SSLR
Day	Pearson Correlation	1	-.191	-.048
	Sig. (2-tailed)		.312	.801
	N	30	30	30
SSLR	Pearson Correlation	-.191	1	.189
	Sig. (2-tailed)	.312		.318
	N	30	30	30
SSLR	Pearson Correlation	-.048	.189	1
	Sig. (2-tailed)	.801	.318	
	N	30	30	30

** . Correlation is significant at the 0.01 level (2-tailed).

Table 7. Pearson's correlation analysis of left and right SSL and parturition day of 6 periparturient Saanen goats

Table 7 showed the SSL Left and Right were not significantly correlated to parturition day with $P > 0.05$ for both sides. The correlation were analysed using Pearson's correlation analysis (parametric test), because the data were normally

distributed. The R value for SSL Left was 0.191 and SSL Right was 0.048. Both showed a weak strength of association to parturition day.

		Day	SSLL	SSLR	P4
Day	Pearson Correlation	1	-.191	-.048	-.706**
	Sig. (2-tailed)		.312	.801	<.001
	N	30	30	30	30
SSLL	Pearson Correlation	-.191	1	.189	-.055
	Sig. (2-tailed)	.312		.318	.773
	N	30	30	30	30
SSLR	Pearson Correlation	-.048	.189	1	-.059
	Sig. (2-tailed)	.801	.318		.756
	N	30	30	30	30
P4	Pearson Correlation	-.706**	-.055	-.059	1
	Sig. (2-tailed)	<.001	.773	.756	
	N	30	30	30	30

** . Correlation is significant at the 0.01 level (2-tailed).

Table 8. Pearson's correlation analysis of P4 concentration, left and right SSL and parturition day of 6 periparturient Saanen goats

Table 8 showed the P4 concentration and SSL Left and Right were not significantly correlated with both $P > 0.05$. However, the R value for P4 concentration and SSL Left was 0.773 and R value for P4 concentration and SSL Right was 0.756, both showed a moderate strength of association.



5.0 Discussion

5.1 Progesterone

The serum progesterone concentration in this study showed a decreasing trend from Day -1 until Day 3 post-partum. The changes between Day -1 to Day 0 was significant ($P < 0.05$) from 3.77 ng/mL to 0.94 ng/mL and changes between Day 0 to Day 1 post-partum was also significant. The results in this study is similar to a few studies done by Singer (2004), Hussain (2016) and Zakar et al. (2007) stating that there will be a significant drop in progesterone level during the day of parturition. The decrement of progesterone became less drastic on Day 2 and Day 3 post-partum.

Serum progesterone concentration also showed significant negative correlation ($P < 0.05$) with a strong strength of correlation with parturition day. The sudden decrease in progesterone level allows estrogen surge and parturition to take place.

The reduction in progesterone concentration is due to lysis of corpus luteum which happens during parturition. Without suppression, the estrogen will surge alongside with relaxin to ease the parturition process. Estrogen is a sex hormone responsible for the development and regulation of female reproductive organ. Estrogen will stimulate the production of prostaglandin (PGF₂α), estradiol (E₂) and increase the receptors for oxytocin (Weiss, 2000). Besides, estrogen also controls the softening of cervix by altering the arrangement and alignment of collagen, elastin and glycosaminoglycans but the mechanism is poorly understood (Weiss, 2000). Both hormones play a crucial role in uterine contractility and cervical dilation during parturition. The shift from progesterone to estrogen dominance environments will

happen gradually, changing the intrauterine environment from a quiescent condition to a dyssynchronous contractions and finally to an active coordinated contractions few hours before term.

During pregnancy, the hormone decreases myometrial contraction by inhibiting myometrial gap junction formation to send signals. Moreover, progesterone also down-regulates the production of $\text{PGF2}\alpha$, development of calcium channels (which is responsible for smooth muscle contractions) and oxytocin receptors (Weiss, 2000). In accordance to our results and a few studies mentioned previously, progesterone withdrawal happens drastically 1 day prior to parturition changing the dynamic of intrauterine environment and initiating the onset of parturition.

5.2 Sacrosciatic Ligament Relaxation

An increasing trend in both left and right sacrosciatic ligament relaxation was observed from Day -1 to Day 0. The sacrosciatic ligament relaxation for both left and right sides peaked on the day of parturition reaching 7.33 mm and 8.17 mm respectively.

In this study, there was a weak strength of association between parturition day and both left and right SSL, but they were not significantly correlated. However, the strength of association between serum progesterone and both left and right SSL was moderate but they were not significantly correlated as well. These results differed from a study done by Shah et al. (2006) stating that there was significant correlation

between parturition day and SSL on both left and right sides. The different results could be due to the small sample size of this study (n=6) while the study done by Shah et al. (2006) used 37 samples. A small sample size may make it difficult to determine if a particular outcome is a true finding and can contribute to Type II error whereby one fails to reject the null hypothesis when it is actually false (Jones, 2003). Even though it is generally acceptable for animal studies to have a small sample size of 5 to 10 due to ethical reasons, the statistical power can be very small (Cristian et al., 2020). Besides that, the parity could potentially play a role in SSL relaxation. Studies have found that primiparous goats are more likely to experience dystocia due to incomplete dilatation of cervix compared to multiparous goats (Sharma, 2015; Majeed et al., 1989). The dilatation of cervix is affected by estrogen surge and so is sacrosciatic ligament relaxation (Shah et al., 2006). However, no study has been done to prove the relationship between parity and sacrosciatic ligament relaxation. All the Saanen goats employed in this study were multiparous, but different parity could be a factor to consider when measuring the relaxation of sacrosciatic ligament. Another factor that could possibly affect the measurement of sacrosciatic ligament is the size of doe but no scientific research has been done as of now. All these factors could lead to an insignificant result that supports the null hypothesis.

6.0 CONCLUSION

In conclusion, this study failed to reject the null hypothesis which states that there is no correlation between sacrosciatic ligament relaxation and serum progesterone concentration in periparturient Saanen goats. However, there was an increasing trend of both left and right sacrosciatic ligament relaxation 24 hours prior to parturition with moderate strength of association. Besides that, P4 concentration is able to predict parturition with a significant drastic drop 24 hours prior to parturition.

7.0 RECOMMENDATION

If similar studies are conducted in the future, it is recommended to have a larger sample size in order to achieve a more valid result with stronger statistical power and reduce the chances of Type II error. Besides that, categorizing the does into different groups based on their parity, size and number of fetus may also enhance the results, as all these factors have not been proven to be mutually exclusive to the extent sacrosciatic ligament relaxation. The frequency and duration of measuring parameters (serum progesterone and sacrosciatic ligament relaxation) can be increased to better track the fluctuations of these parameters, making the results more precise.

REFERENCES

- Abu Bakar, Amie Marini (2014) Genetic characterisations of four goat breeds in Malaysia assessed using microsatellites. Masters thesis, Universiti Putra Malaysia
- Alhaji M, Farhana A. Enzyme Linked Immunosorbent Assay. [Updated 2022 Feb 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK555922/>
- Bhattacharyya, H. K., Bhat, F. A., & Buchoo, B. A. (2015). Prevalence of dystocia in sheep and goats: a study of 70 cases (2004-2011). *Journal of Advanced Veterinary Research*, 5(1), 14-20.
- Bosc, M., Guillimin, P., Bourgy, G., & Pignon, P. (1988). Hourly distribution of time of parturition in the domestic goat. *Theriogenology*, 30(1), 23–33. [https://doi.org/10.1016/0093-691X\(88\)90260-9](https://doi.org/10.1016/0093-691X(88)90260-9)
- Boyazoglu, J., Hatziminaoglou, I., & Morand-Fehr, P. (2005). The role of the goat in society: Past, present and perspectives for the future. *Small Ruminant Research*, 60(1–2), 13–23. <https://doi.org/10.1016/j.smallrumres.2005.06.003>
- Britannica, T. Editors of Encyclopaedia (2018, January 12). Saanen. *Encyclopedia Britannica*. <https://www.britannica.com/animal/Saanen>

Cristian Ricci, Jeannine Baumgartner, Linda Malan & Cornelius M. Smuts (2020)

Determining sample size adequacy for animal model studies in nutrition research: limits and ethical challenges of ordinary power calculation procedures. *International Journal of Food Sciences and Nutrition*, 71:2, 256-264, DOI: 10.1080/09637486.2019.1646714

Crowther, J. R. (2009). *The ELISA Guidebook* (Vol. 516). Humana Press.

<https://doi.org/10.1007/978-1-60327-254-4>

Department of Veterinary Services. (2020). Malaysia: Livestock Statistics

2019/2020. Department of Veterinary Sciences.

<http://www.dvs.gov.my/index.php/pages/view/3338>

Department Of Veterinary Services. (2021). Malaysia: Livestock Statistics

2020/2021. Department of Veterinary Services.

<https://www.dvs.gov.my/index.php/pages/view/3949>

Devendra, C., & Haenlein, G. F. W. (2016). Goat Breeds. *Encyclopedia of Dairy*

Sciences: Third Edition, 1, 77–97. [https://doi.org/10.1016/B978-0-08-100596-](https://doi.org/10.1016/B978-0-08-100596-5.00622-3)

[5.00622-3](https://doi.org/10.1016/B978-0-08-100596-5.00622-3)

Dubeuf, J.P., Morand-Fehr, P., & Rubino, R. (2004). Situation, changes and future

of goat industry around the world. *Small Ruminant Research*, 51(2), 165–173.

<https://doi.org/10.1016/j.smallrumres.2003.08.007>

Gerson W. (2000). Endocrinology of Parturition, *The Journal of Clinical*

Endocrinology & Metabolism, Volume 85, Issue 12, Pages 4421–

4425, <https://doi.org/10.1210/jcem.85.12.7074>

Hussain, S.O., & Coll (2016). Serum progesterone analysis for monitoring estrus , pregnancy and parturition in Shami goats. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 14(2), 69-73.

Ishag, I. A., Abdalla, S. A., & Ahmed, M. K. A. (2012). Factors affecting milk production traits of Saanen goat raised under Sudan-semi arid conditions. *Online Journal of Animal and Feed Research*, 1(5), 435-438.

Jackson, P.G.G. 1995. Handbook of Veterinary Obstetrics. W.B. Saunders Co. Ltd., UK. Pages 109–132.

Jones S.R., Carley S., Harrison M. (2003). An introduction to power and sample size estimation. *Emergency Medicine Journal*. 1;20(5):453–8.

Khandoker, M. A. M. & Syafiee, M. & Rahman, M. S. R. (2017). Morphometric characterization of Katjang goat of Malaysia. *Bangladesh Journal of Animal Science*. 45. 17. 10.3329/bjas.v45i3.31035.

Khanum, S. A., Hussain, M., & Kausar, R. (2007). Assessment of reproductive parameters in female Dwarf goat (*Capra hircus*) on the basis of progesterone profiles. *Animal Reproduction Science*, 102(3–4), 267–275.
<https://doi.org/10.1016/J.ANIREPROSCI.2006.11.010>

Lequin, R. M. (2005). Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA). *Clinical Chemistry*, 51(12), 2415–2418.
<https://doi.org/10.1373/clinchem.2005.051532>

- Liang, J. B., & Paengkoum, P. (2019). Current status, challenges and the way forward for dairy goat production in Asia – conference summary of dairy goats in Asia. *Asian-Australasian Journal of Animal Sciences*, 32(8), 1233–1243. <https://doi.org/10.5713/ajas.19.0272>
- Majeed, A. F., & Taha, M. B. (1989). Dystocia in local goats in Iraq. *Small Ruminant Research*, 2(4), 375–381. [https://doi.org/10.1016/0921-4488\(89\)90032-1](https://doi.org/10.1016/0921-4488(89)90032-1)
- Mcsporrán, K.D. (1980). Dystocia in sheep. In: *Current Therapy in Theriogenology*. Morrow, DA, (2nd Ed.). W.B. Saunders Co., Philadelphia. p. 916-18.
- Mehta, V., Nagar, D. Yadav, R.C., Garg, N. and Purohit, G.N. (2002). Obstetrics problems in goats. 5th National Seminar on Indian Society for Sheep and Goat Production and Utilization, December 30-31, Jaipur, p. 151.
- Mehta, V.M., Deshpande, S.B., Pai, J., Tiwari, S. and Patel, A.V. (1990). Pregnancy and parturition behavior of Surti and Marwari goats. *Indian Journal of Animal Reproduction* 11: 46- 48.
- Mellado, M.; Valdez, R.; Garcia, J.E.; López, R.; Rodríguez, A. (2006). Factors affecting the reproductive performance of goats under intensive conditions in a hot arid environment. *Small Ruminant Research*, 63, 110–118.
- Miller, B. A., & Lu, C. D. (2019). Current status of global dairy goat production: an overview. *Asian-Australasian Journal of Animal Sciences*, 32(8), 1219–1232. <https://doi.org/10.5713/ajas.19.0253>
- Minnesota Veterinary Anatomy Courseware. (2021). <http://vanat.cvm.umn.edu/>

Purohit, Govind Narayan. (2006). Dystocia In The Sheep and Goat-A Review. *Indian Journal of Small Ruminants*, 12, 1-12.

Sánchez-Dávila, F., Barragán, H. B., del Bosque-González, A. S., & Ungerfeld, R. (2018). Social dominance affects the development of sexual behaviour but not semen output in yearling bucks. *Theriogenology*, 110, 168–174. <https://doi.org/10.1016/J.THERIOGENOLOGY.2018.01.004>

Shah, K. D., Nakao, T., & Kubota, H. (2006). Plasma estrone sulphate (E1S) and estradiol-17 β (E2 β) profiles during pregnancy and their relationship with the relaxation of sacrosciatic ligament, and prediction of calving time in Holstein–Friesian cattle. *Animal Reproduction Science*, 95(1–2), 38–53. <https://doi.org/10.1016/J.ANIREPROSCI.2005.09.003>

Shahudin, M.S. & Ghani, A.A.A. & Saad, Mohd & Abu Bakar, Md Zuki & Jesse A, Faez Firdaus & Haron, Abd Wahid & Abu Hassim, Hasliza. (2018). The necessity of a herd health management programme for dairy goat farms in Malaysia. *Pertanika Journal of Tropical Agricultural Science*. 41. 1-18.

Sharma, Amit. (2015). Retrospective Analysis of Dystocia in Small Ruminants. *Intas Polyvet*. 15. 287-289.

Sharma, V.K., Suthar, B.N., Parsani, H.R. and Ojha, S.C. (1999). A clinical study on dystocia in small ruminants. XVth Annual Convention and National Symposium on Biotechniques in Optimizing Fertility in Farm Animals. PAU, Ludhiana, Abstracts, p. 94.

Singer, L., Kumar, M., Gavin, W. & Ayres, S. (2004). Predicting the onset of parturition in the goat by determining progesterone levels by enzyme immunoassay. *Small Ruminant Research*, 52, 203-209. 10.1016/S0921-4488(03)00259-1.

Thorburn, G. D., & Schneider, W. (1972). The progesterone concentration in the plasma of the goat during the estrous cycle and pregnancy. *Journal of Endocrinology*, 52(1), 23–36. <https://doi.org/10.1677/joe.0.0520023>

Zakar, T., & Hertelendy, F. (2007). Progesterone withdrawal: key to parturition. *American Journal of Obstetrics and Gynecology*, 196(4), 289–296. <https://doi.org/10.1016/J.AJOG.2006.09.005>

Zeder, M. A., & Hesse, B. (2000). The initial domestication of goats (*Capra hircus*) in the Zagros Mountains 10,000 Years Ago. *Science*, 287(5461), 2254–2257. <https://doi.org/10.1126/science.287.5461.2254>

APPENDICES

APPENDIX A. Statistical analyses

		95%	Upper
Confidence Interval of the Lower		4.510297	4.730148
Difference			
Std. Error Difference		.755248	.755248
Mean Difference		2.827500	2.827500
	Two-Sided p	.004	.012
	One-Sided p	.002	.006
t-test for Significance			
Equality of Means	df	10	5.362
	t	3.744	3.744
Levene's Test Sig.		<.001	
for Equality of Variances	F	28.053	
Equal variances assumed			
Equal variances not assumed			
P4			

Table 1. Independent samples t-test of P4 concentration between Day -1 to Day 0

Independent Samples Test

	95% Confidence Interval of the Difference	
	Upper	Lower
Mean Difference	.951423	-.960390
Std. Error Difference	.203244	.194277
Mean Difference	.577333	.577333
Two-Sided p	.006	.008
One-Sided p	.003	.004
t	10	8.524
df	3.439	3.439
Levene's Test for Equality of Variances	.090	
F	3.518	
Equal variances assumed		
Equal variances not assumed		

Table 2. Independent sample t-test of P4 concentration between Day 0 to Day 1

Independent Samples Test

		95% Confidence Interval of the Difference	
		Lower	Upper
Std. Error Difference		.062070	.062070
Mean Difference		.061167	.061167
Significance		Two-Sided p	One-Sided p
t-test for Equality of Means		.348	.174
df		10	7.366
t		.985	.985
Levene's Test for Equality of Variances		.108	
F		3.110	
		Equal variances assumed	Equal variances not assumed
P4			

Table 4. Independent samples t-test of P4 concentration between Day 2 to Day 3

		95% Confidence Interval of the Difference		95% Confidence Interval of the Difference	
		Upper	Lower	Upper	Lower
Mean Difference		5.699	-1.699	5.728	-1.728
Std. Error Difference		1.660	1.660	1.660	1.660
t-test for Equality of Means		2.000	2.000	2.000	2.000
t		.256	.258	.113	.130
df		.128	.129	.056	.065
Sig.		1.205	1.205	1.739	1.739
Levene's Test for Equality of Variances		.505	.505	.111	.111
F		.479	.479	3.058	3.058
		Equal variances assumed	Equal variances not assumed	Equal variances assumed	Equal variances not assumed
		SSL	SSL	SSLR	SSLR

Table 6. Independent samples t-test of left and right SSL between Day 0 to Day 1

Independent Samples Test

		95% Confidence Interval of the Difference		95% Confidence Interval of the Difference	
		Upper	Lower	Upper	Lower
t-test for Mean Difference	Std. Error Difference	2.954	2.955	1.207	1.229
Equality of Significance	Two-Sided p	-3.287	-3.289	-1.873	-1.896
Means	One-Sided p				
df		.454	.454	.320	.321
t		10	9.958	10	9.042
Levene's Sig.		-.119	-.119	-.482	-.482
Test for Equality of Variances	F	.902		.156	
		.016		2.358	
	Equal variances assumed	SSLL		SSLR	
	Equal variances not assumed				
	Equal variances assumed				
	Equal variances not assumed				

Table 7. Independent samples t-test of left and right SSL between Day 1 to Day 2

Test	Independent Samples	95% Confidence Interval of the Difference		t-test for Mean Difference	Std. Error Difference	Equality of Variance	Sig.	Levene's Test for Equality of Variances	F	Confidence Interval of the Difference								
		Upper	Lower							Two-Sided p	One-Sided p	Upper	Lower	Two-Sided p	One-Sided p			
Levene's Test for Equality of Variances	SSL	Equal variances assumed	4.287	-1.954	1.400	1.167	.424	.212	10	.833	.902	.016						
		Equal variances not assumed	4.289	-1.955	1.400	1.167	.424	.212	9.958	.833								
t-test for Mean Difference	SSLR	Equal variances assumed	2.302	-2.636	1.108	-.167	.883	.442	10	-.150	.173	2.156						
		Equal variances not assumed	2.383	-2.716	1.108	-.167	.884	.442	8.101	-.150								

Table 8. Independent samples t-test of left and right SSL between Day 2 to Day 3