PREGNANCY CHECK AND FETAL DEVELOPMENT ON INDONESIAN DOMESTIC RABBITS BY MEANS OF ULTRASONOGRAPHY

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BOGOR
2015
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I hereby present the copyright of my undergraduate thesis to Bogor Agricultural University.

Bogor, August 2015

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**ABSTRACT**

CHARISHA FLORENCE FRASER. Pregnancy Check and Fetal Development on Indonesian Domestic Rabbits by Means of Ultrasonography. Supervised by LIGAYA ITA TUMBELAKA and DENI NOVIANA.

This research is carried out to detect the earliest day of conception in Indonesian domestic rabbits by analyzing embryonic vesicle, body diameter and head diameter. The animals used for this research were six pregnant rabbits weighed between 1.75 – 3.90 kg. SonoDop ultrasound system with curved and linear transducers, ultrasound scanner (S-A5V) and Landwind Medical Mirror5 were used. The does were examined on day 5 after mating and every 2 to 3 days until they gave birth. On day 5, the embryonic vesicle size is 10.25 ± 0.96 mm and on day 10, the size is 22.50 ± 0.71 mm. The equation for it is $x = y + 0.2/0.245$. The average body diameter on day 18, 21, 24, and 29 were 0.96 ± 0.049 cm, 1.17 ± 0.025 cm, 1.49 ± 0.049 cm and 1.56 ± 0.026 cm respectively. The equation is $x = y - 0.0021/0.0562$. The average head diameter on day 18, 21, 24, and 29 were 0.79 ± 0.11 cm, 1.02 ± 0.08 cm, 1.22 ± 0.08 cm and 1.59 ± 0.02 cm respectively. The equation is $x = y + 0.5073/0.0723$. All $x$ above is number of days. The earliest day of conception was determined on day 5 in Indonesian domestic rabbits.
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Undergraduate Thesis as a requirement for a degree in Bachelor of Veterinary Medicine Faculty of Veterinary Medicine

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FOREWORD

Praise and grace to the Lord for His guidance and blessings during the writing of this undergraduate thesis. This thesis entitled “Pregnancy Check and Fetal Development on Indonesian Domestic Rabbits by Means of Ultrasonography” was done from January until March 2015 at Pakuan Animal Clinic and Cat Distro, Bogor.

The writer would like to thank everyone who made this undergraduate thesis possible:

1. Dr Drh Ligaya ITA Tumbelaka, SpMP, MSc and Prof Drh Deni Noviana, PhD as supervisors for their assistance and guidance in making this thesis.
2. Ibu Rini Madyastuti Purwono, M.Si, Apt as the academic advisor for assistance and support these past 3 years.
3. Drh Arni and Drh Sabrina for their assistance at Pakuan Animal Clinic and Cat Distro.
4. Father, Archdeacon Charles Fraser, mother, Deva Nesam, brother, Chrisender, cousin sister, Melinda, late grandmother, and my family in Malaysia for their unconditional love and support.

The writer hopes that this thesis will be beneficial to the readers, especially veterinary small animal practitioners.

Bogor, August 2015

Charisha Florence Fraser
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INTRODUCTION

Background

Rabbits are animals that are widely kept as pets, bred for their fur, consumed as meat, and are used for laboratory research. They belong to the order called Lagomorpha (Dallas 2006). Detecting the pregnancy of a rabbit can be done by manual palpation. However, heavy-handed palpation may harm the fetuses and cause distress to the mother (Morrell 1995). Therefore, ultrasonography (USG) was used to detect the pregnancy of a rabbit because it is a non-invasive method. This technique has been found useful to determine the number as well as normal development or loss of fetuses (Ypsilantis and Saratsis 1999).

There have been a number of research done on rabbits using USG such as on reproduction, ophthalmology, muscle and also echocardiograph (Ergin and Besalti 2012). Studies indicate the usefulness of USG as a tool to monitor the success of a breeding program, by determining pregnancy rates and embryonic death (Lamb and Fricke, 2005). The knowledge of the day of conception is required to predict a delivery on the basis of the onset of a gestation (Lacave et al. 2004). Ultrasonography has added benefits such as fetal sexing, early embryonic detection and is less invasive than rectal palpation (Lamb and Fricke, 2005). Besides, it also has the ability to visually characterize the uterus, fetus, ovary, corpus luteum, and follicles (Lamb and Fricke, 2005).

Fetal ultrasound scanning is used routinely in humans and large domestic species for medical, veterinary and animal management purposes in order to monitor fetal growth (Chavatte-Palmer et al. 2008). In order to study the influence of fetal growth on further development in animal models like the rabbit, methods of measurement of fetal and placental size must be measured and viability must be established and validated (Chavatte-Palmer et al. 2008). The examination on the rabbit uterus is performed transcutaneously (Ypsilantis and Saratsis, 1999). Therefore, in this research, USG on pregnant rabbits will be conducted.

Objective

This research is carried out to detect the earliest day of conception in Indonesian domestic rabbits by means of ultrasonography as well as to study the fetal development by analyzing the images produced during the pregnancy check on embryonic vesicle, body diameter and head diameter.

Benefit

To be able to determine the pregnancy detection in Indonesian domestic rabbits using ultrasonography.
LITERATURE REVIEW

Rabbits

The bones of a rabbit are small and fragile and often are destroyed or rearranged by predators and therefore its origin and evolution is difficult to trace (McNitt et al. 2013). The leporids which consist of hares and rabbits appear to almost all breeds of domestic rabbits are descendants of the European wild rabbit, *Oryctolagus cuniculus*. They have said to be originated from Iberian Peninsula (Spain and Portugal) and southern France (McNitt et al. 2013, Taylor et al. 2010).

Below is the taxonomy of a domestic rabbit.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Animalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Chordata</td>
</tr>
<tr>
<td>Class</td>
<td>Mammalia</td>
</tr>
<tr>
<td>Order</td>
<td>Lagomorpha</td>
</tr>
<tr>
<td>Family</td>
<td>Leporidae</td>
</tr>
<tr>
<td>Genus</td>
<td>Oryctolagus</td>
</tr>
</tbody>
</table>

Rabbits were originally classified as rodents but are now placed in a separate order called Lagomorpha. This is because they have two more incisor teeth compared to rodents. They are further divided into two major families, pikas and rabbits and hares. The genera of rabbits can be divided into two, which are the true rabbits (*Oryctolagus*) and the cottontails rabbits (*Sylvilagus*). *Oryctolagus* comprises of the wild European rabbit and its domesticated descendants, which include all the breeds of domestic rabbits. There are 22 chromosomes pairs in *Oryctolagus* (McNitt et al. 2013).

The first recorded rabbit husbandry was in early Roman times, when rabbits were kept in walled gardens (McNitt et al. 2013). Female rabbits are called does, males are called bucks, and neonates are termed “kits”. The particular rabbits that are raised for food are called “fryers”. They generally enjoy good health if kept under sanitary conditions, receive adequate water and nutrition, and are protected from predators, environmental extremes, and trauma (Taylor et al. 2010).

Both development of age are important to be taken into consideration to determine the proper time to start the reproduction of the individual rabbits. Sexually, the smaller rabbits usually mature earlier than the larger breeds. The does that have a moist, swollen, reddish-pink vulva are ready to breed (McNitt et al. 2013).

Rabbits are not native to Indonesia and therefore not many rabbit species can adapt to the tropical temperature. This is also closely related to their physical condition. A few species that were found to be adaptable were New Zealand White, Angora, Rex, Satin, Holland Lop, Flemish Giant, Dutch, Lion and Netherland Dwarf (Yuliarti 2014). The term Indonesian Domestic Rabbits came because of the vast crossbreeding that took place between these species that has brought them to differ in sizes, weight, and physical appearances.
Reproduction in Rabbits

Reproduction refers to the formation of more individuals, from one parent which is known as asexually or from two parents known as sexually. Reproduction is closely associated with protection against adverse conditions and survival over unfavorable periods. Mammal reproduction is by a sexual process and the male and female reproductive anatomy is very similar. Fetal growth rate has been estimated in only some cases (Dallas 2006). Sexual receptivity in rabbits is known to be influenced by androgen (Beyer and McDonald 1973, Boiti C 1998).

Factors such as season, parity, age and weight of females influence the reproductive efficiency of the animals (Kumar et al. 2005). Mammals have the most advanced reproductive systems in the animal kingdom. They have both internal fertilization as well as internal development. The advantage of internal development is that the female mammal does not have to remain in one place, as birds do when incubating their eggs. This leads to a reasonable normal life during pregnancy (Dallas 2006).

Reproductive Anatomy

Ovulation in rabbits are coitus-induced (Taylor et al. 2010, Hill 2012). The female reproductive organs include the ovaries, oviducts, uterus, cervices, vagina and external genitalia. The ovaries, are the primary organ that produces the eggs or ova, and hormones. These hormones include estrogen and progesterone. The ovaries lie within the abdominal cavity, with one on each side, near the kidneys. The ovaries are ovoid structures about 20x10 mm and weigh 0.50-0.75 g, depending on the activity of the ovarian components. The central portion, or medulla, of each ovary consists of connective tissue containing nerves and blood vessels. The outer layer, or cortex, contains the ova in various stages of development, as well as other types of tissue, including blood vessels, nerves, and muscles fibers (McNitt et al. 2013).

Does have duplex uterus with each uterine horn having its own cervix and eight to ten mammary glands (Taylor et al. 2010). The site of fertilization is the oviduct. It functions in the maturation process of spermatozoa known as capacitation and where the early embryonic development occurs. The uterus is the organ in which most of the embryonic development occurs. It also provides muscular force for expulsion of the fetuses at birth. The uterus of the rabbit is formed of two distinct horns, which do not join to form a body and connects into an individual cervical canal, which opens into the common vagina. The cervices function as muscular plugs to keep the uterine horns closed except during mating and parturition. The vagina is the site of sperm deposition at mating and acts as a channel for the young at parturition (McNitt et al. 2013).

Puberty and Estrous Cycle

Rabbits usually mature according to their sizes. The average age that a rabbit reaches sexual maturity are between four and nine months (Merck 2011). The estrus cycle is made up of five stages which depends whether the species is polyestrous or monoestrous. Stage one of the cycle is anestrous where there is no
activity on the part of the reproduction organs. Pro-estrous is the second stage before estrous where follicle-stimulating hormone (FSH) secretion cause follicles to develop in the ovary. FSH stimulates the ovary to release estrogen causing changes to the reproductive tract and preparing for pregnancy. During estrous, ovulation occurs and this causes the FSH levels to decrease and luteinizing hormones to increase and the ripened Graafian follicles to rupture hence releasing the ova. The final stage is metestrous where hormone activity fades and tissues are less active. If the ovum is fertilized, the corpus luteum is formed and it produces progesterone which maintains the pregnancy and estrogen secretion is decreased. The corpus luteum decreases in size which reduces the production of progesterone if pregnancy does not occur (Dallas 2006).

**Mating, Pregnancy and Parturition**

The degree of mating receptivity is indicated by the color of the vaginal orifice and by the amount of moisture on the labia. A doe is most receptive when the vagina is red and moist. Does that are not receptive have a whitish pink vaginal color with little or no moisture (Merck 2011).

After mating, ovulation occurs 10 hours after mating and the ova remain viable for only 6 to 8 hours. Cellular division and development of embryo begin immediately after fusion between the ovum and the sperm. It takes 72 hours before the embryo migrates to the uterus. Pregnancy in rabbits has an average of 28 to 31 days on lesser depending on the breeds. The average litter size obtained is 2 to 7 kits (Dallas 2006). Gestation lengths are generally longer with smaller litters (McNitt *et al*. 2013).

To predict a delivery on the basis of the onset of a gestation requires a knowledge of the day of conception. Visual observation towards the end of gestation can be helpful as there are signs in the pregnant female such as the swelling of the mammary glands, refusing to eat or visible contractions can indicate the onset of parturition though at times it’s unreliable (Lacave *et al*. 2004). This research is performed on Indonesian domestic rabbits because it has not been done before.

**Ultrasonography**

Ultrasonography (USG) examination is a useful, non-invasive way to rapidly evaluate organ health in animals and humans. It is also a rapid method for pregnancy diagnosis, and experienced palpators adapt to ultrasound quickly (Lamb and Fricke 2005). The application of real-time ultrasonography in the study of the anatomy and physiology of the reproductive organs in exotic, wild and endangered species is increasing (Lacave *et al*. 2004).

The diagnosis of USG use ultrasound beams in the range of 2-13MHz (Noviana *et al*. 2012). The sound beam penetrates the patient and reflects off a tissue at acoustic interfaces and returns to the crystal where it is then detected. The sound beam then reflects within tissues at acoustic interfaces. An acoustic interface is where two different tissues are adjacent to each other. This may also mean the capsules surrounding tissues are adjacent structures within organs or
different cells within tissues. The higher the frequency of sound used, the better the resolution of the end image and the more likely small changes will be identifiable. However, higher frequency has lesser penetration (Silkowski 2010). Therefore deeper structures needs a lower frequency.

An ultrasound device consists of a probe and a monitor. There are three types of ultrasound transducers. They are linear, sector/curved, and phased array. Ultrasound imaging is divided into two: Brightness mode (B-mode) and Motion mode (M-mode). B-mode releases multiple ultrasound pulses at all times and is shown as a two dimensional imaging of the organ. Whereas M-mode is the first method of USG that produces views of a moving echo of a heart. B-mode is the best standard imaging that is used in animals to detect pregnancy, tumor infestation or inflammations (Noviana et al. 2012).

The three levels of echogenicity based on the result of USG (sonogram) are hyperechoic, hypoechoic and anechoic. Hyperechoic is a projection with rich and highly reflective echoes, appearing as light grey. Hypoechoic is a projection in a monitor with a lower amount of echoes that appear as shades of dark grey. Anechoic is when there are no echoes and it appears black on screen (Silkowski 2010). Hyperechoic shows high echogenicity which means that the echo produced is bright and is seen as white color in the images produced (Noviana et al. 2012). For example, bone appears black or anechoic on ultrasound with a bright hyperechoic rim. This is because, the beams cannot penetrate bone, and it cast an acoustic shadow beyond it (Ihnatsenka and Boezaart 2010). Therefore, a sonogram view of pregnancy is usually hyperechoic.

**MATERIALS AND METHOD**

**Time and Place**

This study was performed from January until March 2015 at Pakuan Animal Clinic and Cat Distro (PACCD), Bogor.

**Animals and Materials**

The animals used for this research were six pregnant and healthy female Indonesian Domestic rabbits. Some of which were borrowed from a rabbit farm and some were bought for the purpose of this research. The does weighed between 1.75 – 3.90 kg. The rabbits were fed commercial rabbit food and water (ad libithum). The does were kept in a cage with nesting boxes for them to give birth in. They were transported to PACCD every three days once for the ultrasound.

Ultrasound console used for this research was SonoDop ultrasound system, ultrasound scanner (S-A5V) and Landwind Medical Mirror5. A curved and linear transducers with frequencies between 5.5 MHz and 7.5 MHz were also used alongside this research. Other materials such a clipper, shaver, ultrasound gel and tissue were also used in this research.
Ultrasound Examination

The does were examined on day 5 after mating and later, every 2 to 3 days until they gave birth. This examination also depended whenever the USG device was available. Prior to examination, the ventral abdomen towards the pubic area of the doe was clipped and shaved from cranial to caudal. The doe was restrained at dorsal recumbency throughout the examination using USG. The operator needed an assistant to help restrain the doe. The does usually stayed calm and relaxed during the whole examination which took at least half an hour, as long as the environment remained very quiet.

The B-Mode of ultrasonography was used to examine the uterus of the rabbit transcutaneously as seen in Figure 1. The probe is positioned externally against the abdominal wall from right to left with the probe in the sagittal orientation after localisation of the urinary bladder. Prior to scanning, the ultrasound gel is applied at the examination site. The examination moves up from caudal to cranial.

![Figure 1. Ultrasound performed in an Indonesian domestic rabbit transcutaneously](image)

During the first week of gestation period, starting on day 5, a scan to identify and estimate the number of embryonic vesicles were carried out. Complete embryonic vesicle examination was performed on both uterine horns.

The second to fourth week of gestation period, commencing from day 14 until day 30, structures that were identifiable were embryonic vesicle, body diameter and head diameter by ultrasound and was measured from frozen-frame pictures on the monitor using the USG devices. The embryonic vesicle was measured when the largest surface area appeared on screen. The cross-section of the abdomen was measured when two hyperechogenic lines were seen at the sides of the abdomen.

The head diameter was measured from an image when the cerebral falx was clearly visible. Since the wall of the skull was very thin, at a 90° angle with the cerebral falx, the widest distance between the outer borders of the cranium were measured. Observations on the liver, heart, lungs were made occasionally when possible.
Sonogram Interpretation

Parameters of movement that occurred (fetal viability), changes in size, changes in the shape, and change in echogenicity were recorded and analyzed during this research.

Data Analysis

The results measured were the size of the embryonic vesicle, body diameter and head diameter using the average value and is displayed in the form of tables and graphs with the analysis of linear regression equation. Whereas the development of organs in fetuses by descriptive analysis. The data were performed offline using Image-J©.

RESULT AND DISCUSSION

Based on the results obtained, the embryonic vesicles were observed and identified five days after the rabbits mated. The embryonic vesicles could be identified early because the embryo has already implanted in the endometrium wall. These results were later compared to Chavatte-Palmer et al (2008) and Ypsilantis and Saratsis (1999) that embryonic vesicles were seen on day 7. This was not much of a difference because according to El-Gayar et al. (2014), by USG, uterine fluid was detected at an average of six days after mating. Furthermore, as stated in (McNitt et al. 2013), sexually, the smaller rabbits usually mature earlier than the larger breed, therefore it is highly possible that due to the size of the Indonesian domestic rabbit, the embryonic vesicle was seen much earlier. The embryonic vesicle was an anechoic circle which was situated close to the urinary bladder. The position of the probe had to be accurate in order for these vesicles to be seen. It was rather challenging to find these embryonic vesicles due to their small size.

The vesicle size was measured according to the circle diameter in this research. On day 5, the embryonic vesicle size measured at an average of 10.25 ± 0.96 mm and on day 10, the embryonic vesicle doubled its size to 22.50 ± 0.71 mm. The difference in the sizes were due to the increase in development of the embryonic vesicle that was observed.
Figure 2 shows the interrelation between the number of days and the development size of the embryonic vesicle of pregnancy and the standard deviation in Indonesian domestic rabbit using analysis of linear regression.

Though the breed of rabbits used in the literature were different to the ones in this research, but the size of the embryonic vesicle does not vary too far from it. The position of the animal and the direction of the probe must be consistent in order for the embryonic vesicle to be measured accurately.

Table 1. Development of fetal body diameter in Indonesian domestic rabbits using B-Mode USG from day 18 until day 29

<table>
<thead>
<tr>
<th>Days</th>
<th>Body Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0.96 ± 0.049</td>
</tr>
<tr>
<td>21</td>
<td>1.17 ± 0.025</td>
</tr>
<tr>
<td>24</td>
<td>1.49 ± 0.049</td>
</tr>
<tr>
<td>29</td>
<td>1.56 ± 0.026</td>
</tr>
</tbody>
</table>

Table 1 shows the development of the body diameter in the fetus. The average body diameter and standard deviation on day 18, 21, 24, and 29 were 0.96 ± 0.049 cm, 1.17 ± 0.025 cm, 1.49 ± 0.049 cm and 1.56 ± 0.026 cm respectively. The measurements recorded shows the increase in size of body diameter development that was taking place in the fetus throughout this research.
Figure 3 shows the interrelation between the number of days and the body diameter as well as the standard deviation from day 18 until day 29 of pregnancy in Indonesian domestic rabbit using analysis of linear regression. After analyzing the data, a new equation was formulated, $x = y - 0.0021/0.0562$. Y is represented by body diameter and x is the number of days. As seen in the figure below, there is a dramatic change in body diameter from day 18 until day 24 due to the rapid growth of the fetus. Whereas from day 24 till day 29, there is only a gradual change which is constant. The formula generated can be used if the sample size used is almost the same.

Table 2. Development of head diameter in Indonesian domestic rabbits using B-Mode USG from day 18 until day 29

<table>
<thead>
<tr>
<th>Days</th>
<th>Head Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>21</td>
<td>1.02 ± 0.08</td>
</tr>
<tr>
<td>24</td>
<td>1.22 ± 0.08</td>
</tr>
<tr>
<td>29</td>
<td>1.59 ± 0.02</td>
</tr>
</tbody>
</table>

Table 2 shows the development of head diameter in Indonesian domestic rabbits using diagnostic imaging of B-Mode USG from day 18 until day 29. The average head diameter and standard deviation on day 18, 21, 24, and 29 were 0.79 ± 0.11 cm, 1.02 ± 0.08 cm, 1.22 ± 0.08 cm and 1.59 ± 0.02 cm respectively. The head diameter measured increased gradually as they were developing on the same pace.
Figure 4. Interrelation between the number of days and the head diameter and the standard deviation from day 18 until day 29 of pregnancy in Indonesian domestic rabbit using the analysis of linear regression.

Figure 4 depicts the interrelation between the number of days and the head diameter and the standard deviation from day 18 until day 29 of pregnancy using the equation formulated, \( y = 0.0723x - 0.5073 \). Y is represented by head diameter and x is number of days. This equation can be used to determine the head diameter in fetuses once the rabbit goes into day 15 of pregnancy and above and when the sample size is similar. This is due to the development that takes place once the embryo implants itself in the uterus of the mother.
Table 3. Level of echogenicity in the development of Indonesian domestic rabbit fetuses throughout different stages of pregnancy.

<table>
<thead>
<tr>
<th>Days of Pregnancy</th>
<th>Level of Echogenicity</th>
<th>Development Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Anechoic</td>
<td>Embryonic vesicles appear anechoic</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Anechoic</td>
<td>Embryonic vesicle and body diameter observed.</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Hyperechoic</td>
<td>Head diameter that was clearly seen.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypoechoic</td>
<td>Body diameter that was measured when two side lines were seen.</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Hyperechoic</td>
<td>Head diameter that was clearly seen.</td>
<td></td>
</tr>
<tr>
<td>Hypoechoic</td>
<td>Body diameter that was measured when two side lines were seen.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperechoic</td>
<td>Head diameter that was clearly seen.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoechoic</td>
<td>Body diameter that was measured when two side lines were seen.</td>
<td></td>
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</tr>
<tr>
<td>Hyperechoic</td>
<td>Head diameter that was clearly seen.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoechoic</td>
<td>Body diameter that was measured when two side lines were seen.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The placenta and the umbilical attached to the fetus was clearly seen.

**CONCLUSION**

The earliest day of conception was determined on day 5 in Indonesian domestic rabbits using ultrasonography. The gestation age of Indonesian Domestic Rabbits can be defined by formulas using the images of embryonic vesicle, body diameter and head diameter.

**REFERENCES**


BIOGRAPHY

Charisha Florence Fraser was born on October 19th 1990 in Ipoh, Perak, Malaysia. She is the first child of Charles Fraser Devadason and Deva Nesam and has one younger brother, Chrisender Devadason Fraser. She went to Methodist (ACS) high school and graduated in 2008. In the same year, she continued her diploma studies at University Putra Malaysia, Campus Bintulu and graduated in Diploma in Food and Estate Management in 2011. She then entered the Faculty of Veterinary Medicine, Bogor Agriculture University in the same year. During her university years, she joined Persatuan Kebangsaan Pelajar-Pelajar Mahasiswa di Indonesia (PKPMI). She was also active in the faculty’s student organisation, Himpunan Profesi Satwa Liar (HIMPRO SATLI) (2012-2015). She hopes to specialise in wildlife surgery and marine mammal medicine.