Jurnal Kedokteran Hewan December 2018, 12(4):97-100
P-ISSN : 1978-225X; E-ISSN : 2502-5600 DOI: https://doi.org/10.21157/ j.ked.hewan.v12i4.6558

CHANGES IN UTERINE CAPABILITY DUE TO THE INCREASED LITTER SIZE AT 7 WEEKS OF PREGNANCY IN KACANG GOAT

Ridi Arif[1], Fadjar Satrija[1], Adi Winarto[2], Arief Boediono[2], and Wasmen Manalu[2]
[1]Department of Animal Infectious Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor
[2]Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, IPB University, Bogor
*Corresponding author: wasmenmanalu@ymail.com

ABSTRACT

The purpose of this research was to study the changes in uterine capability due to the increased litter size in Kacang goat. In this study, 9 pregnant female Kacang goats were divided into 4 different groups based on litter size, namely 1, 2, 3, and 4. At 7 weeks of pregnancy, the experimental Kacang goats were sacrificed to observe the macro and micro parameters of the uterus. The results showed that the litter size had a quadratic correlation with micro parameters of uterus but it had a linear correlation with macro parameters of the uterus. The variables of uterine glands area, glands lumen area, and cytoplasmic area reached maximum conditions at the litter size of 2.5. Litter size had a linear relationship with the volume, weight, and dimension of the uterus. It can be concluded that the optimal number of litter size in Kacang goat was two offsprings which was proven by the optimal function of the uterus to support fetal development.

Key words: Kacang goat, linear correlation, litter size, quadratic correlation, uterine capacity

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui perubahan daya dukung uterus akibat peningkatan jumlah litter size. Sebanyak 9 ekor kambing kacang betina yang telah dipastikan bunting dibagi ke dalam 4 kelompok berbeda yang masing-masing memiliki litter size 1, 2, 3, dan 4. Pada umur kebuntingan 7 minggu hewan percobaan dikorbankan untuk dilakukan pemeriksaan makro dan mikro uterus. Jumlah litter size yang meningkat memiliki hubungan yang bersifat kuadratik dengan gambaran mikro uterus dan hubungan yang bersifat linear dengan gambaran makro uterus. Variabel mikro uterus yang meliputi luas kelenjar, luas lumen kelenjar, dan luas sitoplasma kelenjar uterus mencapai nilai puncak pada litter size 2,5. Peningkatan litter size memiliki hubungan linear dengan volume, bobot, dan dimensi uterus. Dari hasil penelitian disimpulkan bahwa untuk mendapatkan daya dukung uterus yang optimal terhadap pertumbuhan konseptus maka litter size yang terbaik untuk kambing kacang adalah 2.

Kata kunci: kambing kacang, korelasi linear, litter size, korelasi kuadratik, daya dukung uterus

INTRODUCTION

Pregnancy is an important process in the reproductive cycle. Pregnancy consists of three phases, namely early, middle, and end phase. The Early phase starts from conception, implantation, and embryogenesis. The middle phase starts from embryogenesis until organogenesis, while the end phase starts from the maturation phase until delivery. Every process in pregnancy is regulated by a regulating system which is started and initiated by primary pregnancy hormone (Fowden, 1995; Spencer *et al*., 2012). Every phase of pregnancy is an important process which affects the quality of the offspring.

There are numerous factors which affect the pregnancy period which then influence the quality of the offspring, one of them is the number of offspring in one pregnancy period (litter size). Prolific animals normally tend to have more than one litter size, one of which is Indonesian local livestock, namely Kacang goat (Sodiq and Sumaryadi, 2002). However, the problem that is commonly found among prolific animals is the higher the litter size, the lower the weight of offspring produced. In addition, low birth weight is directly correlated with high postnatal mortality and low survival rate (Gardner *et al.*, 2007).

Low birth weight and high mortality due to the high litter size are strongly associated with maternal uterine capacity (Robinson, 1977). Uterine capability to support fetal growth strongly depends on the quality and quantity of uterine glands. Uterine glands consist of cells that express specific genes to produce essential materials to support the growth and development of conceptus (Bell, 1988; Cooke *et al.*, 2013). The function of the glands is very dominant to maintain the viability of conceptus during the early phase of pregnancy because placenta and blood vessels are not completely formed yet. Moreover, the function of uterine glands to provide nutrition for fetuses is still prominent although the placenta and the blood vessel have already completely formed at 15 weeks of pregnancy (Cheong *et al.*, 2013; Filant and Spencer, 2014).

Lower litter size should allow the uterus to provide better supports to the growth and development embryo and fetus during prenatal growth. This condition can be measured from the changes in the uterine macro and micro variables. Information on the change of uterine capability to support the fetal development due to an increase in litter size is not available yet. Therefore, this study was designed to analyze the relationship between the increased litter size and uterine macro and micro variables. Based on the result, it will be found the optimum litter size that can be supported by the uterus.

MATERIALS AND METHODS

Nine female Kacang goats which had been proven to be pregnant were divided into 4 different groups. Pregnancy examination was performed using ultrasonography (USG) at 7 weeks of pregnancy. The results of USG in the form of the number of conceived offspring were used as a basis to divide the samples into different treatment groups. The results of USG showed that there were 4 different litter sizes, therefore the

samples were divided into 4 different treatment groups, namely female with a litter size of 1, 2, 3, and 4. After USG examination, the pregnant female goats from each group were sacrificed. Uterine macro and micro parameters examination were performed afterward.

The uterine macro parameters were uterine volume, weight, and morphometric measurement, while the micro parameters were histological examination of uterine cell and glands. The morphometric examination consisted of measurements of uterine thickness, uterine length, uterine horn length, body length, and cervical length. Histological examinations were performed for uterine wall thickness, uterine gland number, uterine gland area, gland lumen area, glandular cells nucleus area, and gland cytoplasmic area. The examination was performed using a routine hematoxylin-eosin staining.

Data Analysis

The data acquired were grouped according to female groups with similar litter size i.e. into 4 different litter size groups. The data were analyzed using polynomial contrast analysis to evaluate the correlation between litter size and micro and macro parameters measured. Based on the results found, the linear equation and coefficient of determination were determined.

RESULTS AND DISCUSSION

Correlation between Litter Size and Uterine Micro Variables

The results of average measurement of gland area, lumen area, cytoplasmic area, and uterine glands number in litter size 1, 2, 3, and 4 are presented in Table 1. Along with the increase in litter size in the uterus, the micro image of uterine glands also changed. Afterward, p-values from 3 equations which described the corelation between litter size and micro characteristics of uterine glands are presented in Table 2.

The results in Table 2 showed that the corelation between litter size and micro characteristics of uterine glands was quadratic. The micro parameters having significant correlations with litter size were cytoplasmic area and the lumen of uterine gland. ($P<0.01$). Meanwhile, uterine gland area was only significantly different with an increase in litter size ($P<0.05$). Linear and cubic relation pattern could not sufficiently describe the effect of litter size on the micro image of the uterine gland.

Quadratic relation patterns between litter size and uterine gland micro characteristics are presented in Figure 1. The quadratic relationship between litter size and uterine gland micro characteristics showed that increase in the number of conceived offspring was not always followed by an increase in uterine gland capacity to support prenatal growth. The peak value of uterine gland characteristics in this quadratic linear equation was around litter size of 2.5. In litter size of more than 2.5, the uterine gland characteristic became lower.

Uterine gland characteristics have very important roles to determine uterine capacity to support prenatal growth. Uterine glands have vital functions. Subnormal number or abnormal uterine gland function could result in the failure of pregnancy, even though fertilization was occured (Cooke *et al*., 2013). Uterine glands secrete specific materials which are important in the development of conceptus during which the amount and time of secretion could not be substituted by the other glands (Bazer, 1975; Gray *et al*., 2001; Spencer, 2014).

The values of coefficient of determination of glands area, lumen area, cytoplasmic area, glands number, and nuclear area were 59.7%, 50.5%, 66.4%, 12.8%, and 29.3%, respectively. Based on those values, micro characteristics that changed as litter size increased were gland area, gland lumen area, and uterine gland cytoplasmic area, while gland number and uterine gland nuclear area had no significant change. These results indicated changes in uterine gland cellular activities in response to the change in litter size. In litter size 1, the uterine gland characteristics were seemingly low, while litter size 2 showed the more optimum uterine glands characteristics. In litter sizes 3 and 4, there were decreases in the uterine gland characteristics instead. At a glance, litter sizes 2 and 3 appeared to have similar uterine gland characteristics because the relationship pattern was quadratic. However, litter size 2 tended to be better because it had a higher average of uterine gland characteristics. Furthermore, in litter size 2, the uterine space for fetal development was developed better

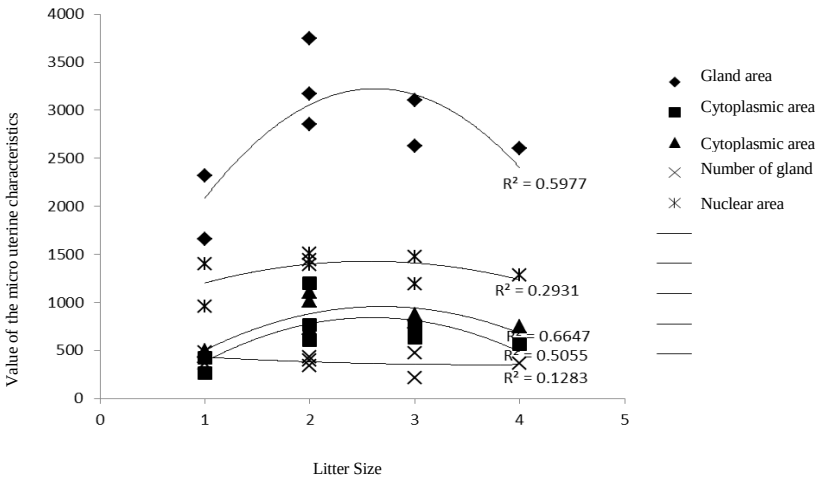

Figure 1. Quadratic relationships between the litter size and uterine gland micro characteristics

Table 1. Average values of gland area (μm^2), lumen area (μm^2), cytoplasmic area (μm^2), and uterine number of gland (gland/mm^2) in litter sizes of 1, 2, 3, and 4

Litter size	Gland area	Lumen area	Nuclear area	Cytoplasmic area	Number of gland
1	1988.10±467.93^{b}	341.94±115.28^{a}	1178.45±307.83^{a}	467.70±44.81^{b}	212.50±40.30^{a}
2	3255.53±451.36^{a}	857.60±306.45^{a}	1446.88±60.47^{a}	951.04±195.39^{a}	195.35±24.30^{a}
3	2865.14±334.13^{ab}	691.33±78.67^{a}	1336.77±200.59^{a}	837.03±54.85^{a}	171.00±91.90^{a}
4	2600.88±459.57^{ab}	566.48±221.29^{a}	1285.04±273.74^{a}	749.35±152.28^{a}	181.00±82.10^{a}

$^{a, ab, b}$Different supercripts within the same column indicate significant difference (P<0.05)

Table 2. P-values of several correlation patterns between the increase in litter size and uterine gland characteristics (number of gland, gland area, gland lumen area, nuclear area, and uterine gland cytoplasmic area)

Relationship pattern type	Variables				
	Number of gland	Area			
		Gland	Lumen	Nuclear	Cytoplasm
Linear	0.586	0.069	0.096	0.433	0.011*
Quadratic	0.754	0.018*	0.009**	0.228	0.009**
Cubic	0.807	0.752	0.684	0.781	0.871

* = Significantly different (P<0.05)
**= Very significantly different (P<0.01)

Table 3. Average values of total uterine weight (g), total uterine volume (mL), uterine lumen area (μm^2), and uterine thickness (x10^{-2} mm) in litter sizes of 1, 2, 3, and 4

Litter size	Weight of uterus + embryo	Volume of uterus + embryo	UL	UT
1	229.21±7.07^{b}	210.25±14.14^{b}	255±57.98^{c}	314.5±13.44^{a}
2	373.25±98.49^{ab}	352.50±95^{ab}	368.25±21.27^{b}	182.5±18.48^{b}
3	633±364.87^{a}	615±332.34^{a}	426±1.41^{ab}	132.5±10.61^{c}
4	457.5±31.82^{ab}	435±21.21^{ab}	490.5±12.02^{a}	140±21.21^{c}

$^{a, ab, b, c}$Different supercripts within the same column indicate significant difference (P<0.05)
UL= Uterine gland lumen area, UT= Uterine wall thickness

Table 4. P-values of correlation patterns between the increase in litter size and uterine macro characteristics

Relationship pattern type	Variables								
	Weight of uterus + embryo	Volume of uterus + embryo	Dimension of uterus			Uterine cornual length		Corpus length	Cervical length
			Length	Width	Thickness	Right	Left		
Linear	0.197	0.178	0.797	0.001**	0.000**	0.544	0.130	0.411	0.607
Quadratic	0.321	0.272	0.507	0.422	0.004**	0.402	0.691	0.120	0.076
Cubic	0.354	0.303	0.365	0.459	0.519	0.701	0.329	0.077	0.958

* = Significantly different (P<0.05)
**= Very significantly different (P<0.01)

compared to litter size 3. These facts showed that uterus had a maximum capacity to support and accommodate the prenatal growth.

Maximum litter size in which the uterus is still capable of providing good supports to maintain the growth and development of conceptus was litter size 2. With litter size of 2, uterine gland micro characteristics appeared more capable of providing nutrition which was showed by the increases in uterine gland area, uterine gland lumen area, and uterine gland cell cytoplasmic area. These better uterine gland characteristics could not be separated from the increase in uterine glands morphogenetic factors, including progesterone, estrogen, IFNT, CSH1, and GH1 (Bartol *et al.*, 1988; Bazer *et al.*, 2012). The increases in uterine gland area showed the increases in gland size and hypertrophic activity, therefore the quality and glandular secretion became more optimal (Igwebuike, 2009). Large uterine gland cytoplasmic area indicated more active synthesis processes. These results increase the secretions of materials required by the fetus in the uterine gland lumen (Nieburgs, 1967). Therefore, uterine gland lumen appeared wider.

Correlation between the Increased Litter Size and Uterine Macro Variables

Changes in uterine macro variables were different from micro characteristics. The results of measurements of total uterine weight, total uterine volume, uterine lumen area, and uterine thickness in litter size 1, 2, 3, and 4 are presented in Table 3. The correlation patterns in the uterine macro parameters tend to be linear rather than quadratic or cubic. The p-values of correlations between uterine macro parameters and litter size are presented in Table 4.

The most evident p-values were found in uterine width and thickness parameters. However, variables of total uterine weight (uterus + embryo) and total uterine volume (uterus + embryo) also tended to have linear correlations. Meanwhile, uterine horn length, uterine body length, and cervical length did not change as litter size increased. The linear relationship between the increase in litter size and uterine macro parameters are presented in Figure 2.

Parameters of total uterine weight and volume only had coefficients of determination of 35.1% and 36.5%, while uterine width and uterine thickness had coefficient

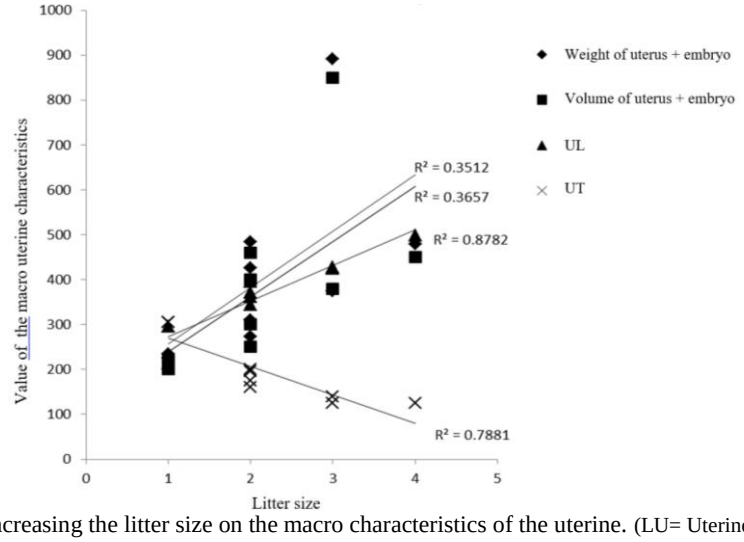

Figure 2. The effect of increasing the litter size on the macro characteristics of the uterine. (LU= Uterine width, TU- Uterine wall thickness)

of determinations of 87.8% and 78.8%. Although the total uterine weight and volume had low coefficient of determinations, the results still showed that an increase in litter size had a direct and linear relationship to uterine weight and volume. The low values of coefficient of determinations were caused by the large variations in uterine weight and volume as litter size increased.

Increase in litter size was automatically followed by the increases in the uterine volume and weight which resulted in the low weight of conceptus in high litter size compared to low litter size (Brooks *et al.*, 1995; Gardner *et al*., 2007). Besides the low weight of conceptus product, the weight variation also increased in a female with a high litter size, which indicates the presence of competition to obtain nutrition among fetuses (Robinson, 1977). These results showed that maternal environment was not sufficient to provide the nutrition required by the conceptus, which subsequently resulted in the low birth weights (Brooks *et al*., 1995; Giussani *et al*., 2003; Gardner *et al*., 2007).

Meanwhile, the uterine dimension (uterine width) also significantly increased along with the increase in litter size. Increase in the uterine width was followed by the thinning of the uterine wall. These conditions could be caused by the increase in the pressure from the lumen of the uterus that will push the uterine wall, therefore the stretching conditions will make the uterine wall thinner.

CONCLUSION

Increase in litter size among Kacang goat has quadratic correlations with uterine micro parameters and linear correlations with uterine macro parameters. The optimal number of litter size in Kacang goat was 2, which was proven by the optimal function of the uterus to support fetal growth and development.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Directorate General of Higher Education, Ministry of Education and Culture for providing the fund through Program Magister Doktor Sarjana Unggul (PMDSU) on the fiscal year of 2016 with contratc number 180/SP2H/LT/DRM/III/2016 on 10 March 2016.

REFERENCES

Bartol, F.F., A.A. Wiley, and D.R. Goodlett. 1988. Ovine uterine morphogenesis: Histochemical aspects of endometrial development in the fetus and neonate. **J. Anim. Sci**. 66(5):1303-1313.

Bazer, F.W. 1975. Uterine protein secretions: Relationship to development of the conceptus. **J. Anim. Sci.** 41(5):1376-1382.

Bazer, F.W., G. Song, J. Kim, K. Dunlap, M. Satterfield, G. Johnson, and G. Wu. 2012. Uterine biology in pigs and sheep. **J. Anim. Sci. Biotechnol**. 3(1):23.

Bell, S.C. 1988. Secretory endometrial/decidual proteins and their function in early pregnancy. **J. Reprod. Infertil**. 36:109-125.

Brooks, A.A., M.R. Johnson, P.J. Steer, M.E. Pawson, and H.I. Abdalla. 1995. Birth weight: Nature or nurture? **Early. Hum. Dev**. 42(1):29-35.

Cheong, Y., C. Boomsma, C. Heijnen, and N. Macklon. 2013. Uterine secretomics: A window on the maternal-embryo interface. **Fertil. Steril**. 99(4):1093-1099.

Cooke, P.S., T.E. Spencer, F.F. Bartol, and K. Hayashi. 2013. Uterine glands: Development, function and experimental model systems. **Mol. Hum. Reprod**. 19(9):547-558.

Filant, J. and T.E. Spencer. 2014. Uterine glands: Biological roles in conceptus implantation, uterine receptivity, and decidualization. **Int. J. Dev. Biol**. 4(164):107-116.

Fowden, A.L. 1995. Endocrine regulation of fetal growth. **Reprod. Fertil. Dev**. 7(3):351-363.

Gardner, D., P. Buttery, and M. Symonds. 2007. Factors affecting birth weight in sheep: Maternal environment. **Reproduction.** 133(1):297-307.

Giussani, D.A., A.J. Forhead, D.S. Gardner, A.J.W. Fletcher, W.R. Allen, and A.L. Fowden. 2003. Postnatal cardiovascular function after manipulation of fetal growth by embryo transfer in the horse. **J. Physiol**. 547(1):67-76.

Gray, C.A., F.F. Bartol, B.J. Tarleton, A.A. Wiley, G.A. Johnson, F.W. Bazer, and T.E. Spencer. 2001. Developmental biology of uterine glands. **Biol. Reprod**. 65(5):1311-1323.

Igwebuike, U.M. 2009. A review of uterine structural modifications that influence conceptus implantation and development in sheep and goats. **Anim. Reprod. Sci**. 112(1-2):1-7.

Nieburgs, H.E. 1967. **Nuclear/Cytoplasmic Ratio (N/C) and Nuclear Chromatin**. **Diagnostic Cell Pathology in Tissue and Smears**. Grune & Stratton, New York & London.

Robinson, J.J. 1977. The influence of maternal nutrition on ovine foetal growth. **Proc. Nutr. Soc**. 36(1):9-16.

Spencer, T.E., K.A. Dunlap, and J. Filant. 2012. Comparative developmental biology of the uterus: insights into mechanisms and developmental disruption. **Mol. Cell. Endocrinol**. 354(1-2):34-53.

Spencer, T.E. 2014. Biological roles of uterine glands in pregnancy. **Semin. Reprod. Med**. 32(5):346-357.

Sodiq, A. and M.Y. Sumaryadi. 2002. Reproductive performance of Kacang goat and Peranakan Etawah goat in Indonesia. **J. Anim. Prod**. 4(2):52-59.