



Timing of fertile period for successful pregnancy in American Bully dogs



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ABSTRACT

Determination of the timing of the estrus cycle is essential for fertile mating. There are physiological variations among breeds, between bitches, and between cycles of the same bitch. If serial monitoring and many tools are applied, the exact moment of ovulation could be pin-pointed. However, it leads to time and costly difficulties. Progesterone concentrations during estrus cycles follow a specific pattern, and it is largely used in timing of fertile period. Although it has similar pattern in general, it is likely that breed-specific differences exist. The aim of this study was to investigate the way of timing the fertile period for successful pregnancy in American Bully dogs based on vaginal cytology and progesterone assay with minimized cost. To identify the empirical relations among reproductive characteristics, we performed statistical analyses on data from proestrus-to-estrus 27 American Bully dogs referred for 7 months. We found the significant correlations between the cyclic changes of vaginal cytology and progesterone assay. The relationship of serum progesterone concentrations with the days from vaginal discharge onset was analyzed through linear regression assay. In conclusion, we addressed two standards in the timing of fertile period with a minimal number of progesterone assays in the breeding management of American Bully dogs.

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1. Introduction

Timing of fertile period is key to increase the success rate of pregnancy in dogs. It is difficult to determine the optimal mating time because of the spontaneous ovulation characteristics of dog. There are many clinical assessments discussed for estimating the optimal window of breeding, such as observation of physical and behavioral signs, vaginal cytology, reproductive hormone measurements, vaginal endoscopy, ultrasonography, and examination of cervicovaginal secretion [1].

Of these methods, progesterone assay has been a valuable tool for assessing the reproductive events in bitches [2]. The preovulatory rise in serum progesterone due to preovulatory follicular luteinization is one of the unique features in canine reproduction.

After LH surge, the serum progesterone rises from around 1 ng/mL during anestrus and early proestrus [3] to 4–10 ng/mL at ovulation [4,5]. Generally, progesterone can be determined either by quantitative or semi-quantitative methods. Semi-quantitative progesterone assays allow for rapid house testing of canine blood samples, however, a subjective component cannot be excluded due to the nature of these tests [6]. Radioimmunoassay (RIA) or Enzyme immunoassay provide accurate and reliable results, but these have the disadvantage of high expense and long turn-around time [7].

Vaginal cytology is another valuable tool that can support a breeding program. It can be used to define the stage of the estrus cycle because the vaginal epithelium undergoes morphologic changes under estradiol and LH transition. Although vaginal cytology has advantages of low cost and availability, it alone is inadequate for accurate timing. Some bitches demonstrate poor cellular changes in the vaginal smear [8] and cornification peaks are variable 1–6 days before the LH surge [3]. Based on the accuracy of progesterone concentration measurement with RIA and easy, but inaccurate aspects of vaginal cytology, we considered the timing of

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fertile period in American Bully dogs with close monitoring of vaginal cytology and necessary times of progesterone RIA. To decide the optimal point of progesterone assay, the relationships between vaginal cytology and progesterone, and between vaginal discharge onset and progesterone were statistically analyzed.

Meanwhile, the American Bully dog is recently accepted as a new breed and has been recognized by the United Kennel Club since 2013 [9]. There is limited reproductive information on this breed yet. Therefore, we hope this study may contribute to the breeding management in American Bully dogs.

2. Materials and methods

2.1. Animals

Twenty-seven clients owned, proestrus to estrus American Bully dogs, presented to the referring hospital for breeding management to decide the mating date from June to December in 2016, became the subjects of study. Detailed information for each subject is described on Table 1. Although their pedigree information is unavailable, the possibility of inbreeding cannot be excluded because of the small pool of American Bully dogs in South Korea. The pregnancy check-ups and delivery through cesarean section of subjects were done in local hospitals due to each owner's preference. Therefore, we gathered the pregnancy progress via contact afterwards. Litter size was defined as the number of puppies alive at birth.

Table 1
Subjects' information

Practitioner	Patient I.D.	Age (Mo.)	Order of estrus (No.)	Previous pregnancy history (No.)	P4 conc at the decision making (ng/mL)	Delivery outcome (litter size)	Etc.
I	A	27	4	2	6.15	3	
	B	38	5	1	6.2	3	
	C	24	2	1	4.41	7	vaginal prolapse
	D	24	3	0	3.46	3	
II	E	36	5	0	5.1	10	
	F	40	5	4	1.23	miscarriage	2 miscarriages in previous pregnancy
	G	48	5	2	2.95	9	Natural delivery
	H	24	5	0	4.4	7	
	I	30	4	0	6.2	1	
	J	38	4	1	1.03	10	
	K	38	4	1	2.57	9	
	L	23	4	1	6.58	5	vaginal prolapse
	M	27	4	2	6.8	5	
	N	24	3	0	3.52	9	
	O	24	2	0	3.9	5	
	P	18	2	1	4.41	5	
	Q	15	2	1	2.99	2	
	R	13	1	0	4.4	4	
	S	9	1	0	0.846	0	Slow rise in preovulatory serum P4 conc
	T	23	1	0	6.14	0	obese
	U	11	1	0	8.4	1	
V	12	1	0	6.5	miscarriage	Miscarriage due to Car accident	
W	19	1	0	12.8	1	obese	
X	14	1	0	5.82	2		
Y	9	1	0	6.1	5		
Z	11	1	0	2.57	9		
a	36	4	0	8.85	0	surgical AI, vaginal polyp and pyometra	

Twenty seven American Bully dogs became the subjects of the study. The day when the progesterone (P4) level initially reached 4 ng/mL or more was regarded as the day of ovulation. Some of mating date decision were made earlier than 4 ng/mL because the clinical visits were available only on workdays. Therefore, the mating was decided as two days after the P4 level of 1–2 ng/mL; one day after the P4 level of 2–4 ng/mL; the very day when the P4 level reaches 4 or higher. Additional mating was recommended two days after the initial mating.

Other information that may affect the fertility are described on the "Etc." column. Besides breeding determination, further check-ups and delivery were taken at local hospitals. Miscarriages were diagnosed in local hospital also. Except for "a", vaginal artificial insemination (AI) were done by one breeder who owns male American Bully dog. Except for "G", patients delivered through Cesarean section.

The male American Bully dog, which became the sole semen donor in this study, is owned by a breeder. Its fertility including clearance of *Brucella canis* were examined in local hospital periodically. Mating was performed through vaginal insemination with fresh semen by the breeder, except for one dog (a) which had surgical insemination.

The numbers of animals was differentially allocated according to the statistical design.

- 1) Forty blood and vaginal smear samples of 20 bitches (E ~ X) were used to examine the correlations between serum progesterone concentration and vaginal cytology indices. Vaginal cytology indices are 'cornified cell ratio' and 'cornification index'. To exclude the performer's bias, these 20 bitches are selected because their assessments were conducted by one clinician.
- 2) Fifty blood and vaginal smear samples of 24 bitches (A ~ X) were used to categorized and analyzed for the Chi-square test. Categorizing procedure was considered to redeem the inter-practitioners bias.
- 3) Thirty-nine samples of 15 dogs were used for a linear regression assay to predict the progesterone concentration based on the day from vaginal discharge onset. Subjects with single visit (n = 6), inconclusive detection on vaginal discharge (n = 4), and possible reproductive disorders (n = 2; S,T) were excluded. However, data of "a" were included in this analysis, in spite of its pyometra and vaginal polyp which were discovered in

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