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COMPARISON OF ULTRASONOGRAPHIC MEASURES OF REPRODUCTIVE TRACT IN THAI SWAMP BUFFALO HEIFERS AND COWS

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ABSTRACT

Although artificial insemination (AI) technology is widely used in buffalo breeding in Thailand, AI in buffaloes has a lower conception rate compared to AI in beef cattle. It is crucial to understand the development of the ovary and reproductive system of buffalo heifers and cows. The objective of the present study was to evaluate the reproductive tract characteristic using ultrasound measurements during the luteal and follicular phases in Thai swamp buffalo heifers and cows. The studies of reproductive tract were conducted in buffalo cows (n=8; BCS=3.38) and heifers (n=8; BCS=3.25). The reproductive tract was evaluated by measuring the size of the ovary, dominant follicle, CL, uterine horn, cervix, and vulva. The buffalo cows had larger diameter of the cervix and vulvar width (P<0.05) compared with those of heifers. In the follicular phase, buffalo cows had a mean ovarian diameter (ipsilateral POF) of 2.35 cm, and heifers had a mean ovarian diameter of 2.24 cm (P>0.05). The average preovulatory follicle diameter was 1.29 cm in buffalo cows and 1.18 cm in heifers (P>0.05). In the luteal phase, the mean ovarian diameter (ipsilateral CL) of buffalo cows was significantly larger than heifers (P<0.05; 2.46 vs. 2.09 cm, respectively). The diameters of the CL in buffalo heifers and cows were 1.27 and 1.47 cm, respectively (P>0.05). Buffalo cows and heifers have no differences in ovarian components in the follicular and luteal phases, but there are differences in the size of the reproductive tract, which reproductive tract of buffalo cows is larger than heifers.

Keywords: *Bubalus bubalis*, buffaloes, Thai swamp buffalo, reproductive tract, ultrasonography, ovarian component

INTRODUCTION

The livestock production in Thailand has a growing interest and emphasis on raising buffaloes to increase their population and enhance the buffalo breed. To expand and breed animals, the livestock production industry employs artificial insemination (AI) technology. As a consequence, the development of techniques and equipment

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impact the success of artificial insemination. Although artificial insemination is fast and highly efficient for beef cattle (Bó *et al*., 2007; Dahlen *et al*., 2014; Lamb *et al*., 2016), but the conception rate in Thai swamp buffalo is relatively low (Chaikhun *et al*., 2010). The reasons that artificial insemination is unsuccessful may be due to the optimize AI timing and fertility of female buffaloes. The estrus expression in the buffalo has been poor or silent heat, and it has a longer estrus expression than cattle (Mondal *et al*., 2007; Chaikhun *et al*., 2010; Yindee *et al*., 2011). The standing heat is the best reliable sign to estimate the optimized AI timing that relates to ovulation time in both cattle and buffaloes (Thakur *et al*., 2013). In the field of female cattle and buffalo reproduction examination, ultrasonography has a higher accuracy compared to rectal palpation. Furthermore, the data or report on the reproductive tract of the female Thai swamp buffalo is quite limited, especially the data from the ultrasound examination. Generally, the use of real-time ultrasound in bovine breeding has advanced rapidly in the last decade, that technology is useful for reproductive examination and assist in performing AI, embryo transfer (ET), pregnancy diagnosis, identify characteristics of uterine disorders and uterine involution. Ultrasonography is a non-invasive examination of an animal reproductive tract that can be performed without impairing fertility or having a negative effect on reproductive system or conception (Kahn, 1992; Ribadu and Nakao, 1999; Fontes and Oosthuizen, 2022). For the most part, the great advantage of the animal reproductive ultrasound may accomplish the understanding of female buffaloes reproductive system and further successfully improve the AI. Thus, the present study was aimed to evaluate the reproductive tract characteristics and ovarian component using ultrasound measurements during the luteal and follicular phases in Thai swamp buffalo heifers and cows.

MATERIALS AND METHODS

Location and animals

This study was carried out in the stable of the buffalo and beef cattle farm, Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand, at the coordinates LS 13°49′14″and LO 100°3′45″, with an average annual temperature of 24 to 37ºC on the summer season from March to July. The study used 16 animals selected based on uniformity of age and body condition score from the herd (80 heads of swamp buffalo). Thai swamp buffalo heifers (n=8; BCS≥3.0, 1 to 5 scale according to the method described by Alapati *et al*., 2010) and multiparous non-suckling cows (n=8; BCS≥3.0) were selected and used in the present study. Animals raised in pens (5x5 m; 2 animals/pen), fed mainly on Para grass (*Brachiaria mutica*), and supplemented with concentrate to meet nutritional requirements according to weight, production, and physiological state, and fresh water was supplied *ad libitum*. The present study was approved by the committee on animal experiments of the Kasetsart University, Thailand, following the guidelines of animal care and use under the ethical review board of the office of the national research council of Thailand (ACKU64-AGR-018).

Estrus detection

Estrus was detected twice a day in the morning (6:00 to 7:00 h) and evening (16:00 to 17:00 h) by using penile translocation buffalo bull (Figure 1) and behavior was recorded. The critical observation of estrus behavior such as mounting, clear mucus discharge from the vulva and standing heat was conducted.

Assessment of reproductive parameters by real-time ultrasound

The measurement of the width of vulva and the length of the rima (according to the method described by Mesquita *et al*., 2016) (Figure 2) were conducted by using a Vernier caliper (RS PRO 150 mm, digital caliper) in the follicular phase (at standing heat time) and luteal phase (8 days after standing heat).

In the follicular phase, the diameter and thickness of the cervix and uterine horn, the dominant or preovulatory follicle (DF or POF; diameter, cm; area, cm^2) and ovarian size ipsilateral DF (diameter, cm; area, cm^2) were measured using ultrasound (SonoScape, Medical Corp.) by a transrectal probe via a B mode screen at standing heat time. In the luteal phase, the diameter and thickness of the cervix and uterine horn, corpus luteum (CL; diameter, cm; area, cm^2) and ovarian size ipsilateral CL (diameter, cm; area, cm^2) were measured using ultrasound (SonoScape, Medical Corp.) by a transrectal probe via a B mode screen at day 8 after standing heat detection.

Statistical analysis

Data in vulvar width, rima length, cervical diameter, cervical thickness, ovary diameter and area, DF or POF diameter and area, CL diameter and area were taken to verify the correctness. Before analysis, the data distribution was evaluated by descriptive statistics, including mean, minimum, maximum, frequency, and standard deviation. The reproductive parameters of buffalo heifers and cows were tested, which comparing the differences between the means of the experimental heifers vs. cows by T-test using SAS OnDemand program (Institute, 2014).

RESULTS AND DISCUSSIONS

Body condition score (BCS) and body conformation of Thai swamp buffalo heifers and cows

Body condition score and body conformation of Thai swamp buffalo heifers and cows (Table 1) did not show a statistical difference. The Thai swamp buffaloes that were selected in this study had the same uniformity and BCS in both heifers and cows. The BCS of buffalo heifers was 3.25 while the BCS of cows was 3.38, BCS is a tool used to assess the amount of metabolizable energy stored in fat and muscle in domestic animals. The BCS can be used to determine the energy status for nutrition or feeding management, this is a crucial factor in the productivity and reproductive performance of cattle cows (Gearhart *et al*., 1990; Pryce *et al*., 2001) and buffalo cows (Alapati *et al*., 2010).

Vulvar width and rima length in the follicular and luteal phases of Thai swamp buffalo heifers and cows

Vulvar width and rima length are shown in Table 2. The results suggested that vulvar width of buffalo cows was significantly larger than heifers in follicular phase ($P<0.01$; 8.35 vs 5.95 cm, respectively) and luteal phase ($P<0.05$; 7.90 vs 5.35 cm, respectively). There were no significant differences in the rima length between buffalo heifers and cows in follicular and luteal phases. Previously studied (Mesquita *et al*., 2016) was reported that the larger vulva relative to body size were good judges of the ovarian follicular reserve in cattle. Additionally, further investigation into the associations between vulvar size and fertility in Thai swamp buffaloes may be required for further animals selection and herd management.

The ultrasonography measurement of cervical and uterine horn diameter in the follicular and luteal phases of Thai swamp buffalo heifers and cows

The ultrasonography measurement of cervical and uterine horn diameter is shown in Table 3. In luteal phase, the cervical diameter of buffalo cows was significantly larger than heifers ($P<0.05$; 2.12 vs 1.32 cm, respectively). Generally, cervical dilation and increase in diameter after parturition (Van Engelen *et al*., 2007). It was previously reported that the cervical diameter of mature water buffaloes was 1.5 cm (Nguyen *et al*., 2014), while the information on ultrasonography measurement of cervical and uterine horn in swamp buffalo is quite limited. There were no significant differences in the diameter of uterine horn between buffalo heifers and cows in follicular and luteal phases.

The ultrasonography measurement of ovary size and component in the follicular and luteal phases of Thai swamp buffalo heifers and cows

The representative ultrasonography of Thai swamp buffalo is shown in Figure 3. The measurement of ovary size and component in the follicular and luteal phases of Thai swamp buffalo heifers and cows are shown in Table 4 and Table 5, respectively. In follicular phase, there were no significant differences in the diameter and area of ovary and POF between buffalo heifers and cows. The diameter of ovary ipsilateral POF in buffalo heifers and cows were 2.24 and 2.35 cm, respectively and POF diameter in buffalo heifers and cows were 1.18 and 1.29 cm, respectively. These results were similar to previous studies reported that ovary and POF diameter of swamp buffaloes were 2.07 cm and 1.55 ± 0.24 cm (Nguyen *et al*., 2014) and water buffaloes were 3.0 cm (Singh *et al*., 2000) and 1.30 to 1.60 cm (Baruselli *et al*., 1997). Buffalo cows had a significantly larger diameter of the ovary ipsilateral CL during the luteal phase than heifers ($P<0.05$; 2.09 and 2.46 cm, respectively), while the diameter and area of CL were not significantly different between buffalo heifers and cows. The diameter of CL in buffalo heifers and cows were 1.27 and 1.47 cm, respectively. These results were similar to previous studies reported that CL diameter of water buffaloes was 1.00 to 1.70 cm (Baruselli *et al*., 1997; Drost, 2007).

In conclusion, this study suggested that Thai swamp buffalo cows and heifers have no difference in ovarian components during follicular and luteal phases, but there are differences in the size of the reproductive tract, which reproductive tract of buffalo cows is larger than those of heifers. Taken together, these reproductive tract ultrasound measurement results of female Thai swamp buffalo could be beneficial in improving AI and understanding the reproductive system.

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Figure 1. Estrus detection with penile translocation buffalo bull.

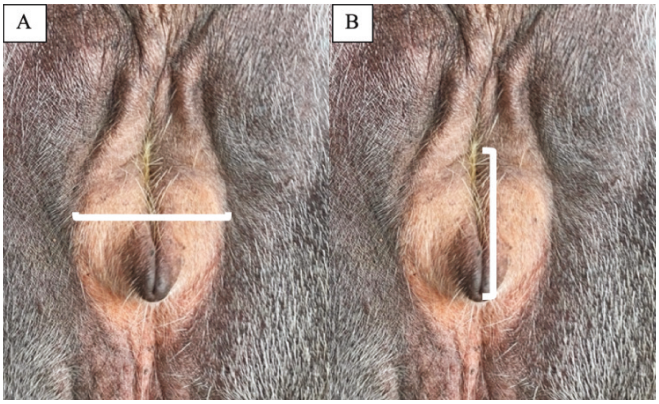

Figure 2. Measure the width of the vulva (A) and the length of the rima (B) in buffalo.

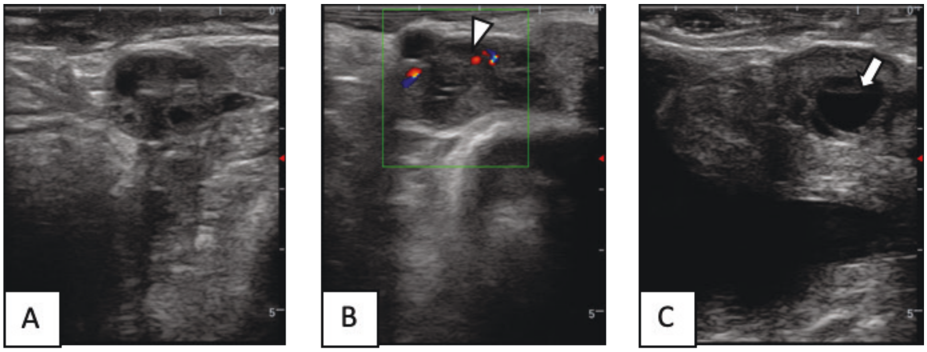

Figure 3. Representative ultrasonography of Thai swamp buffalo ovary (A), CL with open head arrow (B) and preovulatory follicle with open arrow (C).

Table 1. Body condition score (BCS), body conformation and vulvar measurements of Thai swamp buffalo heifers and cows.

Items	Heifer	Cow	SEM	P-value
Number of animals, n	8	8		
BCS (1-5 scale)	3.25	3.38	0.101	0.554
Body conformation				
Shoulder height, cm	139.63	135.75	1.079	0.071
Hip height, cm	135.13	134.88	0.342	0.728
Body length, cm	152.75	150.75	1.456	0.511
Heart girth, cm	213.50	211.00	0.946	0.196

Note: SEM (Standard Error of Mean).

Table 2. Vulvar width and rima length in the follicular and luteal phases of Thai swamp buffalo heifers and cows.

Items	Heifer	Cow	SEM	P-value
Number of animals, n	8	8		
Vulvar width (cm)				
Follicular phase**	5.95	8.35	0.339	<0.001
Luteal phase*	5.35	7.90	0.364	0.019
Rima length (cm)				
Follicular phase	6.53	7.65	0.326	0.123
Luteal phase	6.28	7.55	0.347	0.098

Note: SEM (Standard Error of Mean), *indicated a significant difference between means within a row P<0.05, **indicated a highly significant difference between means within a row P<0.01.

Table 3. The ultrasonography measurement of cervical and uterine horn diameter in the follicular and luteal phases of Thai swamp buffalo heifers and cows.

Items	Heifer	Cow	SEM	P-value
Number of animals, n	8	8		
Diameter of cervix (cm)				
Follicular phase	1.41	2.14	0.178	0.058
Luteal phase*	1.32	2.12	0.184	0.046
Diameter of the right uterine horn, cm				
Follicular phase	1.32	1.51	0.066	0.313
Luteal phase	1.29	1.47	0.069	0.222
Diameter of the left uterine horn (cm)				
Follicular phase	1.29	1.39	0.055	0.422
Luteal phase	1.22	1.37	0.067	0.313

Note: SEM (Standard Error of Mean); *indicated a significant difference between means within a row $P<0.05$.

Table 4. The ultrasonography measurement of ovary diameter and area in the follicular phase of Thai swamp buffalo heifers and cows.

Items	Heifer	Cow	SEM	P-value
Number of animals, n	8	8		
Diameter (cm)				
Right ovary	2.14	2.34	0.090	0.305
Left ovary	2.01	2.02	0.074	0.901
Ovary ipsilateral POF	2.24	2.35	0.084	0.575
POF	1.18	1.29	0.049	0.291
Area (cm^2)				
Right ovary	3.23	3.62	0.272	0.510
Left ovary	3.29	3.40	0.307	0.871
Ovary ipsilateral POF	3.51	4.18	0.234	0.174
POF	1.08	1.24	0.100	0.465

Note: SEM (Standard Error of Mean), *indicated a significant difference between means within a row $P<0.05$, POF; preovulatory follicle.

Table 5. The ultrasonography measurement of ovary diameter and area in the luteal phase of Thai swamp buffalo heifers and cows.

Items	Heifer	Cow	SEM	P-value
Number of animals, n	8	8		
Diameter, cm				
Right ovary	2.18	2.45	0.095	0.180
Left ovary	2.02	2.03	0.060	0.921
Ovary ipsilateral CL*	2.09	2.46	0.070	0.026
CL	1.27	1.47	0.053	0.171
Area, cm^2				
Right ovary	3.52	3.83	0.135	0.304
Left ovary	3.32	3.50	0.200	0.648
Ovary ipsilateral CL	3.31	3.84	0.125	0.081
CL	1.25	1.44	0.079	0.498

Note: SEM (Standard Error of Mean), *indicated a significant difference between means within a row P<0.05. CL; corpus luteum.

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