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การศึกษาช่วงวัยเจริญพันธุ์ของกระบือปลักสาวภายใต้ระบบการเลี้ยงแบบผสมผสานในภาคตะวันออกของประเทศไทย

ธัชฎาพร ไชยคุณ-มาร์คูว[1,#] และ อารยา สืบขำเพชร[2]

[1]คลินิกสูติศาสตร์ ไกเน่วิทยา แอนโดรวิทยาและเทคโนโลยีชีวภาพสัตว์ คณะสัตวแพทยศาสตร์
มหาวิทยาลัยเทคโนโลยีมหานคร กรุงเทพมหานคร ประเทศไทย
[2]สาขาวิชาพรีคลินิก คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมหานคร กรุงเทพมหานคร ประเทศไทย

บทคัดย่อ: การเลี้ยงกระบือฟาร์มพ่อแม่พันธุ์บางฟาร์มในประเทศไทยในปัจจุบันได้พัฒนาเป็นระบบการเลี้ยงแบบผสมผสาน โดยมีการเสริมอาหารข้น การจัดการที่ลดภาวะความเครียด การผสมพันธุ์กับพ่อพันธุ์ที่ได้รับการยอมรับว่ามีพันธุกรรมดีเยี่ยม และการจดบันทึกข้อมูลอย่างเป็นระบบ เพื่อเป็นการต่อยอดประโยชน์รูปแบบการเลี้ยงดังกล่าวงานวิจัยนี้มีวัตถุประสงค์เพื่อทำการศึกษาอายุกระบือปลักสาวที่เหมาะสมต่อการสืบพันธุ์ โดยใช้วิธีการสุ่มตัวอย่างแบบหลายขั้นตอนเพื่อคัดเลือกกระบือปลักเพศเมียจำนวน 25 ตัว จากฟาร์มกระบือปลักพ่อแม่พันธุ์ 6 ฟาร์ม ที่อยู่เขตพื้นที่ภาคตะวันออกของประเทศไทย แบ่งกลุ่มสัตว์ออกเป็นทั้งหมด 4 กลุ่มตามข้อมูลวันเกิด ได้แก่ กลุ่ม 1 อายุ 18-23 เดือน กลุ่ม 2 อายุ 24-29 เดือน กลุ่ม 3 อายุ 30-35 เดือน และกลุ่ม4 อายุ 36 เดือนขึ้นไป ตรวจร่างกายทั่วไป ได้แก่ คะแนนความสมบูรณ์ของร่างกาย น้ำหนักตัว ความกว้างของกระดูกเชิงกรานทั้ง pin bone และ hook bone ร่วมกับการตรวจความสมบูรณ์พันธุ์ เช่น เส้นผ่านศูนย์กลางคอมดลูก โครงสร้างบนรังไข่ และระดับฮอร์โมนโปรเจสเตอโรนในกลุ่มตัวอย่าง 2 ครั้ง ระยะห่าง 14 วัน กระบือที่มีวงรอบการเป็นสัดที่ปกติจะต้องมีระดับฮอร์โมนโปรเจสเตอโรน ≥1 นาโนกรัมต่อมิลลิลิตรอย่างน้อยหนึ่งครั้งในช่วงทำการศึกษา ผลการวิเคราะห์ทางสถิติจากจำนวนสัตว์ที่มีวงรอบการเป็นสัดในแต่ละกลุ่มพบว่า กลุ่ม 2 กระบือปลักสาวอายุ 24-29 เดือน เป็นอายุที่เหมาะสมต่อการสืบพันธุ์อย่างมีนัยสำคัญยิ่งทางสถิติ อย่างไรก็ตามไม่พบความแตกต่างทางสถิติของค่าที่วัดจากการตรวจร่างกายทั่วไปและการตรวจความสมบูรณ์พันธุ์ในแต่ละกลุ่มตัวอย่าง ข้อมูลการลดอายุวัยเจริญพันธุ์ที่น้อยที่สุดที่เหมาะสมต่อการนำกระบือปลักเป็นแม่พันธุ์ที่เลี้ยงในระบบฟาร์มแบบผสมผสาน อาจนำมาช่วยในการสร้างแนวทางจัดโปรแกรมการผสมพันธุ์ (การผสมด้วยน้ำเชื้อจากพ่อพันธุ์ที่ผ่านการคัดเลือก) พัฒนาการจัดการฟาร์ม (การเสริมอาหารข้น) รวมถึงช่วยเพิ่มอายุขัยในการให้ผลผลิตในกระบือได้

คำสำคัญ: *Bubalus bubalis* กระบือสาว กระบือปลัก วัยเจริญพันธุ์ โปรเจสเตอโรน

[#]ผู้รับผิดชอบบทความ
สัตวแพทย์มหานครสาร. 2567(2) 88-102.
E-mail address: thuchadaporn@mut.ac.th

A Study on the Age Range of Puberty of Swamp Buffalo Heifers under an Integrated Farming System in Eastern Thailand

Thuchadaporn Chaikhun-Marcou[1,#] and Araya Suebkhampet[2]

[1]Obstetric- Gynecology Andrology and Animal Biotechnology Clinic, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand

[2]Pre-Clinical Veterinary Science Department, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand

Abstract: Recently, in Thailand, some swamp buffalo breeders have been developing an integrated farming system that incorporates concentrate supplementation, stress-reducing management practices, insemination with proven high-performance bulls and systematic data recording. To facilitate and optimize this process, this study aimed to determine the appropriate age for puberty and reproduction in young swamp buffaloes. Twenty-five swamp buffaloes from six breeder farms in Eastern Thailand were muti-stage sampling selected. The animals were divided into four age groups based on their birthdate records: Group I (18-23 months), Group II (24-29 months), Group III (30-35 months) and Group IV (36 months and older). Physical examinations, including body condition score, body weight and measurements of the pin bone and hook bone, as well as breeding soundness evaluations (including cervical diameter, ovarian structures and progesterone levels), were conducted over a 14-day interval. Cycling buffaloes were identified by a progesterone level (P4) of ≥1 ng/ml at least once during the study period. Statistical analysis of the number of cycling buffaloes in each group revealed that the optimal puberty period for suitable reproduction in swamp buffalo heifers occurred in Group II (24-29 months), with a statistically significant difference noted (P<0.01). However, no statistically significant differences were observed among the groups regarding physical and breeding soundness parameters. This information on reducing the appropriate puberty age for swamp buffalo raised under an integrated farming system may assist in establishing guidelines for breeding programs (utilizing proven bull semen), improving farm management (through concentrate supplementation) and enhancing the lifespan and productivity of buffaloes.

Keywords: *Bubalus bubalis*, heifer, swamp buffalo, puberty, progesterone

[#]Corresponding author J. Mahanakorn Vet. Med. 2024(2) 88-102.

E-mail address: thuchadaporn@mut.ac.th

Introduction

In Thailand, most swamp buffaloes were traditionally raised through free grazing and natural mating. Limitations on the reproductive performance of swamp buffalos are a vital issue affecting farm productivity and is influenced by many factors. Although reproductive physiological traits may frequently be seen as fundamental, delays in the onset of puberty and poor breeding management in young heifers may likewise impede growth and fertility (Chaikhun *et al*., 2010; Warriach *et al*., 2015). According to Jainudeen and Hafez (1993), the age of puberty ranged from 18 to 46 months, influenced by factors such as breed, farm management practices and whether the buffalo had achieved 60% of its adult body weight (250-400 kg). Failure to adequately monitor and record buffalo data is an additional problem, making it challenging to identify the precise age of puberty and the initiation of fertility. Age estimates are frequently based on teething, potentially differing by about six months from the actual age. Previous reports indicated that the age of puberty in Thai swamp buffalo ranged from 30 to 35 months (Intraramongkol and Faree, 2009) similar to Vietnamese swamp buffalo (34-36 months) (Barile, 2005). The age at first calving was 42 months or upon the shedding of the first pair of baby teeth (Intraramongkol and Faree, 2009).

Currently, the buffalo population in Thailand totals 1.8 million, representing an increase of nearly 50% over the past five years (Department of Livestock Development, 2024). Some swamp buffalo breeders are developing an integrated farming system that utilizes concentration supplementation, stress-reducing management, heifers conceived through natural mating or artificial insemination with high-performance bulls, along with data recording to improve fertility and productivity. Depending on farm management, the puberty period for optimal fertility in buffalo heifers may differ significantly from previous findings. In this study, data on age, body weight and reproductive characteristics were analyzed to determine the ideal age range within puberty for optimal fertility in swamp buffalo heifers within the integrated farming management system.

Materials and Methods

The experimental procedures involving animals were approved by the Mahanakorn University of Technology Animal Care and Use Committee in accordance with the university's regulations and policies on laboratory animal care and use. (ACUC-MUT-2023/001).

Animals

The muti-stage sampling was used in the study. The farms were located in Eastern Thailand with the inclusion criteria included concentrate supplementation, stress-reducing management (such as insect prevention, heat stress reduction, private stables and free grazing). Six developed breeder farms were convenience sampling selected. Then twenty-five swamp buffalo heifers with birthdate records and born from artificial insemination or natural mating with proven bulls were randomly collected from each farm. The animals were divided into 4 groups by age in months from the birth date records as follows: group I: 18-23 months (n=6), group II: 24-29 months (n=6), group III: 30-35 months (n=7), and group IV: over 36 months (n=6; with has an age range of 36-61 months). The maximum two heifers from each farm were used in each group.

Physical and breeding soundness examination

The physical and breeding soundness examinations, such as body condition score (BCS), body weight (Raungprim *et al*., 2021) and pin bone and hook bone measurements, were performed by the same observer and the same protocol (Chaikhun-Marcou *et al*., 2017). Cervical diameter and ovarian structures were examined by ultrasonography (Sono V6 RFID, Sonowin®, China). The plasma progesterone level (P) tests were performed by chemiluminescence immunoassay (BRIA Lab, Bangkok, Thailand) to determine breeding soundness, over a 14-day interval. Cycling buffaloes were identified when P4 levels were ≥1 ng/ml at least once, which indicates that the buffalo presented the ovulated corpus luteum in the estrous cycle (Terzano *et al*., 2012; Day and Nogueira, 2013).

Statistical analysis

The number and percentage of cycling heifers were analyzed in each group and compared using the Pearson Chi-square test. The average body condition score, body weight, pin bone diameter, hook bone diameter, cervical diameter and ovarian sizes were determined for each group and compared between groups by the One-way ANOVA test (with normal distribution). Correlations between these parameters were analyzed using the Pearson Correlation test with a 95% confidence interval.

Results

The present study found by a statistically significant margin ($P<0.01$), that the puberty period for optimal fertility in swamp buffalo heifers was group II: 24-29 months. In group II, the average P4 level in cycling buffaloes was 4.81±0.64 ng/ml, which was higher than the others. The average P4 level in all cycling animals was 3.92±0.41ng/ml, while in non-cycling animals it was 0.42±0.08 ng/ml (Table 1).

The mean±SEM of body condition score, body weight, pin bone diameter, hook bone diameter, cervical diameter and ovarian sizes were analyzed in each group and compared between groups using One-way ANOVA. Differences in BCS, body weight, pin bone diameter, hook bone diameter, cervical diameter and right ovarian size were not statistically significant in our study, with the exception of left ovarian size (P=0.002), as shown in Table 2.

The correlation of physical and breeding soundness examination parameters in our study are presented in Table 3. In the present study, the BCS not only showed a positive relationship with body weight but also with hook and pin bone diameters. The right and left ovarian size data from our study suggest a positive correlation (P<0.05), which means that both sides of the ovary are usually of similar size.

Discussion

Puberty period

The present study found, by a statistically significant margin (P<0.01), that the puberty period for optimal fertility in swamp buffalo heifers was group II: 24-29 months. The puberty age of the buffalos was determined by the onset of normal ovulation, ovarian functioning and the estrous cycle (Gupta *et al*., 2016). Our results were similar to a previous study of Thai swamp buffalo, which found an average puberty onset age of 24-25 months (Kamonpatana *et al*., 1987) – but not another, more recent study, which reported a range of 30-35 months (Department of Livestock Development, 2006) and Vietnamese swamp buffalo (34-36 months) (Barile, 2005). However, both previous reports did not mention farming management. This age range of puberty, moreover, can vary widely from country to country. In Bulgaria, the Murrah heifer had an average puberty onset age of 14.2±3.5 months (Viladimir *et al*., 2017) – whereas in India it has been reported as high as 31.53±0.88 months (Haldar and Prakash, 2005). In Italy, the puberty age of Mediterranean heifer was 19.34 months

Table 1. The plasma progesterone levels (mean±SEM) in cycling and non-cycling heifers across different age groups were compared using the Pearson Chi-square test.

Group	Age (month)	Cycling (P_4>1ng/ml)		Non-cycling (P_4<1ng/ml)		Total (N)
		N	Mean±SEM	N	Mean±SEM	
1	18-23	1	3.1	5	0.37±0.06	6
2	24-29	6	4.81±0.64*	0	-	6
3	30-35	6	4.19±0.76*	1	0.24	7
4	≥36	5	2.68±0.56*	1	0.83	6
Total			3.92±0.41		0.42±0.08	25

*Difference in superscript letter within column represent significant differences between groups (P<0.01)

(Terzano *et al*., 2007) – closer, but still significantly lower than our studies average range of 24–29 months. These differences in puberty onset periods in buffalo may be related to differences in breed, feed quality, farm management and other seasonal and/or climate-related factors. Our research was conducted on the modern breeding farms that prioritized animal welfare, utilizing concentrated feed and high-quality genetic resources. These factors likely improved the health and reproductive development of the buffalos in our study, which may explain the differences between our findings and those reported in other studies in Thailand.

The average P4 level in all cycling animals was 3.92±0.41 ng/ml, while in non-cycling animals, it was 0.42±0.08 ng/ml. Our P4 level results were comparable to those of cycling Indian Murrah heifers, which showed 4.27±0.23 ng/ml (Haldar and Prakash, 2005). In contrast, cycling Bulgarian Murrah heifers presented P4 concentration levels lower than those in our study, measuring 1.23±0.16 ng/ml (Viladimir *et al*., 2017).

Onset of puberty is the result of dominant follicle estradiol -17β feedback control on gonadotropin-releasing hormone in the hypothalamus and luteinizing hormone in the anterior pituitary gland responding, which leads to first ovulation (Day and Nogueira, 2013). Progesterone is a key regulator of the estrous cycle, depending on the presence of the corpus luteum (the primary progesterone producer) in the ovary (Terzano *et al*., 2012). Our study found a positive relationship between P4 levels and the presence of the corpus luteum in cycling heifers.

Table 2. The results (mean±SEM) of physical and breeding soundness examinations were determined for each group and compared between groups using a one-way ANOVA.

Group	Age (month)	BCS	BW (kg)	Pelvic (Hook) (cm)	Pelvic (Pin) (cm)	Cervical Diameter (cm)	Ovary (Right) (cm)	Ovary (Left) (cm)
1	18-23	3.33±0.17	483.90±50.41	53.83±2.40	15.75±0.93	1.92±0.83	1.67±0.11	1.45±0.05
2	24-29	3.92±0.20	583.48±40.50	59.33±2.30	18.83±1.94	2.42±0.15	2.12±0.13	2.38±0.13
3	30-35	3.64±0.14	509.37±38.46	56.07±1.26	13.28±0.94	2.50±0.29	2.00±0.18	2.00±0.26
4	≥36	3.75±0.11	560.43±61.60	58.42±2.65	16.16±1.62	2.25±0.11	2.20±0.20	2.50±0.16
Mean±SEM		3.66±0.08	533.30±23.80	56.88±1.10	15.90±0.77	2.28±0.10	1.98±0.88	2.03±0.12
P-value (Between groups)		0.104	0.464	0.308	0.069	0.174	0.121	0.002[a]

[a] Difference in superscript letter within column represent significant differences between groups (*P*<0.05)

Table 3. The correlation of physical and breeding soundness examination parameters was tested by Pearson Correlation.

Parameters	BCS	BW	Pelvic (hook)	Pelvic (pin)	Cervical diameter	Ovary (right)	Ovary (left)
BCS	1	0.673**	0.666**	0.449*	0.318	0.369	0.423
BW	0.673**	1	0.895**	0.577**	0.048	0.534*	0.331
Pelvic (hook)	0.666**	0.895**	1	0.616**	0.074	0.334	0.305
Pelvic (pin)	0.449*	0.577**	0.616**	1	0.069	0.128	0.165
Cervical diameter	0.318	0.048	0.074	0.069	1	-0.095	0.302
Ovary (right)	0.369	0.534*	0.334	0.128	-0.095	1	0.719**
Ovary (left)	0.423	0.331	0.305	0.165	0.302	0.719**	1

* Correlation is significant at the 0.05 level (2-tailed)

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

Interestingly, some heifers were found to be pregnant when their reproductive status was assessed during the first visit (group II: n=2, group III: n=1, and group IV: n=2). The owners were unaware of when the animals were mated, which could result from accidental mating by young bulls in the herds during grazing or mudding times. This information suggests that heifers in group II, aged 24-29 months, could represent the youngest fertile puberty period for swamp buffalo heifers, coinciding with the onset of puberty (Kamonpatana *et al.*, 1987). In our study, no upper age limit was imposed for heifers in the inclusion criteria. Previous research has identified infertility in aging mares, attributed to factors such as poor oocyte quality and uterine condition (Cuervo-Arango *et al.*, 2019). As a result, only one heifer (aged 61 months) was investigated to determine the maximum age at which infertility may occur in heifers under field conditions.

Intrinsic factors associated with determining the optimal period in puberty for fertility in buffalo heifers

Heifers receiving optimal nutrition and achieving proper weight gain before breeding age show improved reproductive performance in their first breeding time and a reduced age of puberty onset (Moorey and Biase, 2020). In our study, the average BCS of swamp buffalo heifers was 3.66±0.08, which is suitable for ovarian function on puberty onset. Additionally, previous data suggest that buffalo heifers should reach approximately 60% of the body weight of mature animals (250-400 kg) by the time of puberty to be suitable for breeding, further highlighting the role of proper nutritional management (Warriach *et al*., 2015; Patel *et al*., 2020; Plansky and Dimitrov, 2020). In our study, feeding with concentrate supplements enabled 24-29 months heifers to reach over 60% of the bodyweight of mature animals (583.48±40.50 kg compared to 800 kg). Achieving this body weight is linked to a reduction in the age of puberty onset and feeding with concentrate supplements can be especially beneficial in promoting early puberty during seasons when high-quality natural roughage is scarce (Afroz *et al.*, 2000; Saadullah *et al*., 2020). In the present study, BCS showed a positive relationship not only to bodyweight but also to hook and pin bone diameters. The optimal puberty age for fertility requires both ovarian cyclicity and parturition ability for healthy pregnancy and calving. Dystocia is the main issue in heifers and pelvic diameter measurement needs to be considered before breeding. There were no statistically significant differences in hook (56.88±1.10 cm) and pin (15.90±0.77 cm) bone diameters in any age group in our study. This result could be assumed that all groups have the similar pelvic structure which might relate to the birth canal size. Previous to this, no studies have examined this link between pelvic diameter and dystocia in buffalo.

A cervical diameter of more than 2.5 cm has been suggested for artificial insemination in buffalo (Chaikhun-Marcou *et al*., 2017). In the age groups involved in our study, there was no statistically significant difference in the cervical diameter, which had an average of 2.28±0.10 cm. Natural mating is suggested for these heifers. Ovarian size in matured buffalo was 2.2-2.6x1.1-1.8x1.1-1.4 cm3 which is smaller than studies on cattle (Singh *et al*., 2000). Our study found the average right and left ovarian size was 1.98±0.88 cm vs 2.03±0.12 cm with no statistically significant difference in any age group (P>0.05) – except for one animal in which the left ovary was larger than right. The majority of studies, however, report the right ovary as usually more developed and mature as well as presenting more follicles (Khandoker *et al*., 2011).

Extrinsic factors associated with determining the optimal period in puberty for fertility in buffalo heifers

The research was conducted between February and May (summer season), with temperatures ranging from 24.8°C to 33.6°C and an average of 28.7°C, accompanied by moderate rainfall (Climatological Center, Meteorological Department, 2024). The temperature in the eastern region was within the favorable range for buffalo farming. Furthermore, the animals were provided with similar feed and feeding protocols and stress reduction in accordance with the inclusion criteria. These conditions were not considered confounding factors influencing the onset of puberty in buffaloes in Eastern Thailand. Similarly, the study of Nili-Ravi buffalo in Pakistan also reported that calves born in winter had a shorter age at puberty which could be the combination effect of season and climatic factors (Zaidi and Anwar, 2021). In tropical countries, the weather and roughage quality are affected by the seasons - so concentrate supplements and feed management may be an alternative way to maintain reproductive functioning and overall health in non-favorable seasons.

In contrast, in non – tropical counties, increased levels of melatonin (found in seasons with shorter periods of daylight) have been reported to induce a 70% increase in reproductive cyclicity as compared with buffalo undergoing long daylight seasons or living in countries in which long daylight periods predominate (Plansky and Dimitrov, 2020). Heat stress also lowers production and reproductive performance in buffalo due to reduced appetite, lowered blood circulation of progesterone and follicular stimulating hormones and high prolactin concentrations (Ahmad and Tariq, 2010). Reducing of those effects from heat stress have been applied by farm management (such as timed water sprinkle, high sealing barn, fan ventilation and swamp bathing) in every farms in our study. Bypass fat and area specific mineral mixture supplement had been improved fertility in peripartum buffaloes (Tripathi and Mehta, 2022) which could be a future study in heifer.

Traditionally, Thai swamp buffalo have been risen by free grazing without any supplements. Nowadays, many breeder farms tend to use free grazing in combination with concentrate supplement feeding in the barn (with and without mosquito protecting nets) similar to the integrated farming system in this study (Chaikhun *et al*., 2012). A comparative study between stable raising and free grazing has reported no difference in the puberty onset age between these two management systems (Sabia *et al*., 2014) but this similarity does not necessarily imply a similar onset or rate of fertility. A combination of these systems may be the ideal management model for both animal welfare and economic efficiency.

Genetic selection is the main tool for improving the overall buffalo population and for enhancing production related and economically desirable traits. Recently research found the age of puberty in beef was important reproductive trait which trended to higher heritability than dairy cattle. However, reproductive traits are limited in buffalo for age at first calving and calving interval traits (Shao *et al*., 2021). In swamp buffalo, physical and reproductive development of the buffalo stock has depended mainly on breeder farms which control the genetics on both the bull and cow sides. Increased growth rates and carcass quality are the main genetic goals of these breeders because of these traits moderate to high heritability which can be transferred by artificial insemination or natural mating with merit bulls (Moorey and Biase, 2020). The heifers in our study were all daughters of merit bulls (the bulls have been approved by the Thailand Department of Livestock Development for breeding and productive performances) and this genetic fact may help explain the difference between our structural and reproductive data from that of previous reports. In addition, potential genetic factors specific to each heifer may have influenced the non-cycling in aging animals and the cycling in young animals observed in this study.

Conclusion

Twenty-five buffalo heifers, bred from merit bulls and fed with concentration supplementation under an integrated farming system, were selected for this study. Our findings on the appropriate age of puberty for fertility in swamp buffalo heifers were 24-29 months (group II in our study), which indicating a statistically significant difference (*P*<0.01). Group II animals, moreover, had 60% of body weight compared to the standard adult weight, with a satisfactory body condition score (3.5/5) and no difference in pin bone and hook bone sizes from group III and IV. This study suggests that recording and monitoring buffalo biological data (e.g., birthdate, estrous cycle, body condition score (BCS), weight, pelvic diameter, and other relevant factors) is essential for determining the optimal time to initiate the breeding process, as well as for maintaining and maximizing the reproductive lifespan and overall health of swamp buffalo. The information from this study might be useful for heifer selection and management for a breeding program without veterinary service on reproductive performance examination in both conventional and integrated farms.

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