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Original Article

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Abstract

The objective of this study was to elucidate ovarian follicular dynamics in swamp buffalo cows *(Bubalus bulalis)* following a protocol for estrus synchronization during hot as low breeding season (March to June) and cool as high breeding season (November to February). Nine pluriparous buffalo cows received a progesterone ear implant for 10 days together with the administration of a luteolytic dose of PGF2α at the time of implant removal to start the estrus cycle. Human Chorionic Gonadotrophin was administered whenever ovulation did not occur within the first five days following implant removal. Daily ultrasound monitoring and blood collection for progesterone values were performed starting one day following implant removal. The progesterone monitoring was used to confirm ovulation and to determine luteal function after ovulation. Data analysis was carried out at least for two consecutive cycles in each cycling buffalo in 22 estrus cycles. It was found that 5/22 (22.7%) were characterized by one wave and 17/22 (77.3%) by two waves of follicle development. Within cycles characterized by one wave of follicle development, emergence was recorded on day 2.3±0.5 and 1.8±0.4 during the low and high breeding season, respectively (p>0.05). Day of estrous cycle when the dominant follicle attained the largest size during the low and high breeding season was 13.5±1.2 and 12.6±1.5, respectively (p>0.05). Largest size (mm) of the dominant follicle during the low breeding and high season was 14.5±2.1 and 16.4±2.7, respectively (p>0.05). Within cycles with two follicular waves during the low breeding season, first and second wave emerged on day 1.2±0.3 and on day 11.4±0.8, respectively, and largest diameter of second wave dominant follicle (10.3±1.2 mm) was recorded on day 19.7±1.1. During the high breeding season, first and second wave emerged on day 0.9±0.4 and on day 10.7±0.9, respectively and largest diameter of second wave dominant follicle (12.8±1.2 mm) was recorded on day 18.9±1.7. Pooling data for both 1- and 2-wave cycles and seasons, mean progesterone value at estrus and ovulation was 0.07±0.03 ng/ml, but after ovulation mean progesterone increased to 2.37±0.5 ng/ml at the mid luteal phase and later, decreased at the late luteal phase which was 0.98±0.2 ng/ml. In conclusion, results from this study showed the first time of the typical pattern of follicle wave development in swamp buffalo.

Keywords : estrous cycle, ovarian follicle development, swamp buffalo

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บทคัดย่อ

การพัฒนาของฟอลลิเคิลในแม่กระบือปลักหลังการปรับขนานการเป็นสัด *(Bubalus bubalis)*

เอกชาติ พรหมดิเรก[1] Giorgio Antonio Presicce[2] Fabio De Rensis[3] จินดา สิงห์ลอ[1] มงคล เตชะกำพุ[1,*]

จุดประสงค์ของงานวิจัยนี้เพื่อศึกษาการพัฒนาของฟอลลิเคิลในวงจรการเป็นสัดของแม่กระบือปลักในฤดูร้อนที่มีความสมบูรณ์พันธุ์ต่ำ (มีนาคมถึงมิถุนายน) และฤดูหนาวที่มีความสมบูรณ์พันธุ์สูง (พฤศจิกายนถึงกุมภาพันธ์) ใช้แม่กระบือปลักที่เคยให้ลูกมาแล้วจำนวน 9 ตัว ที่ได้รับการเหนี่ยวนำให้เป็นสัดเริ่มต้นพร้อมกันด้วยฮอร์โมนโปรเจสเตอโรนชนิดฝังหู ตรวจการพัฒนาของฟอลลิเคิลด้วยเครื่องเรียลไทม์ บี โมด อัลตราซาวน์ และเก็บเลือดตรวจระดับฮอร์โมนโปรเจสเตอโรนทุกวันตั้งแต่วันที่เอาฮอร์โมนโปรเจสเตอโรนออก เพื่อตรวจสอบยืนยันการตกไข่และติดตามการทำงานของคอร์ปัส ลูเตียม หลังเกิดการตกไข่อย่างน้อย 2 รอบวงจรการติดต่อกันเป็นจำนวนทั้งหมด 22 รอบ ผลจากศึกษาได้ยืนยันการพัฒนาของฟอลลิเคิลในกระบือมีลักษณะเป็นคลื่น โดย 5/22 (22.7%) มีลักษณะเป็น 1 คลื่น ในขณะที่ 17/22 (77.3%) มีลักษณะเป็น 2 คลื่น สำหรับวงจรการเป็นสัดที่เป็น 1 คลื่น พบว่าการเริ่มต้นของคลื่นฟอลลิเคิลในฤดูร้อนและฤดูหนาวไม่แตกต่างกัน โดยจะเริ่มในวันที่ 2.3±0.5 และ 1.8±0.4 (p>0.05) ในขณะที่พบโดมิแนนท์ฟอลลิเคิลในวันที่ 13.5±1.2 และ 12.6±1.5 (p>0.05) โดยมีขนาดเท่ากับ 14.5±2.1 และ 16.4±2.7 มม. ในฤดูร้อนและฤดูหนาวตามลำดับ (p>0.05) สำหรับวงจรการเป็นสัดที่เป็น 2 คลื่น ในฤดูร้อนการเริ่มต้นของคลื่นที่ 1 จะพบในวันที่ 1.2±0.3 และคลื่นที่ 2 ในวันที่ 11.4±0.8 โดยโดมิแนนท์ฟอลลิเคิลในคลื่นที่ 2 มีขนาดเท่ากับ 10.3±1.2 มม. พบได้ในวันที่ 19.7±1.1 ส่วนในฤดูหนาว การเริ่มต้นของคลื่นจะเกิดในวันที่ 0.9±0.4 สำหรับคลื่นที่ 1 และคลื่นที่สองในวันที่ 10.7±0.9 ตามลำดับ โดมิแนนท์ฟอลลิเคิลมีขนาด 12.8±1.2 มม. พบได้ในวันที่ 18.9±1.7 จากรวมข้อมูลทั้งสองคลื่นฟอลลิเคิลพบว่าในช่วงที่เกิดการเป็นสัดและตกไข่จะมีค่าเฉลี่ยของฮอร์โมนโปรเจสเตอโรนท่ากับ 0.07±0.03 นาโนกรัม/มล. แต่เมื่อหลังจากเกิดการตกไข่ พบว่าค่าเฉลี่ยของฮอร์โมนโปรเจสเตอโรนในช่วงกลางของระยะลูเตียลมีค่าเฉลี่ยสูงขึ้นถึง 2.37±0.5 นาโนกรัม/มล. และจะลดต่ำลงในช่วงปลายระยะลูเตียล โดยมีค่าเฉลี่ยเหลือเท่ากับ 0.98±0.2 นาโนกรัม/มล. งานวิจัยนี้แสดงให้เห็นเป็นครั้งแรกว่าการพัฒนาของฟอลลิเคิลในรอบของวงจรกระบือปลักมีลักษณะเป็นคลื่น

คำสำคัญ : วงรอบการเป็นสัด การพัฒนาของฟอลลิเคิลในรังไข่ กระบือปลัก

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Introduction

The continual follicle development within an estrous cycle progressing as sequential waves, encompasses the recruitment of small follicles, a common growth phase and the final selection of the dominant follicle together with the regression of other follicles. Studies on follicular dynamics have extensively been performed in cattle (Sirois et al., 1988; Knopf et al., 1989; Ginther et al., 1989; Sunderland et al., 1994; Lean et al., 1992) whereas only few reports are available in the buffalo species (Presicce, 2006). Mostly, studies have been performed in riverine buffaloes, both in calves and adult animals. Presicce et al. (2003) reported in Mediterranean buffalo calves had a similar pattern of follicle development as previously described by Adams et al. (1994) in cattle calves. In a study by Baruselli et al. (1997) on follicular dynamics in Murrah buffaloes, 1 to 3 waves of follicle development were recorded and the number of waves was found to be linked to the luteal phase and length of the estrous cycle, as previously described in cattle (Ginther et al., 1989). Swamp buffaloes are characterized by estrous cycles of variable length and variable time interval between the end of estrus and ovulation (Bodhipaksha, 1987). Such peculiar reproductive aspects can similarly be found in riverine buffaloes as well, being characterized by reproductive efficiency affected by the duration of day length, together with a high rate of silent estrus and seasonal anestrus (Zicarelli, 1997). Swamp buffaloes' ovarian activity may be characterized by a seasonal low breeding season that last from April to July (summer season) during which estrus cycles are reduced or absent while from November to January (cool season), the reproduction performance is higher. However, no report on follicular dynamics during these two periods has been found. Within this framework, a more in-depth understanding of follicular turnover in swamp buffaloes in the course of favorable (high breeding season) and unfavorable season (low breeding season) could help to improve reproductive function and efficiency, especially in the areas of synchronization of ovulation for artificial insemination and embryo transfer, as well as superovulatory treatments. The aim of this study was therefore to evaluate for the first time the follicular dynamics of swamp buffaloes following an estrus synchronization protocol.

Materials and Methods

Animals: Nine pluriparous swamp buffalo cows, 3 to 10 yrs old (means = 5.4) and weighing between 400 and 600 kg (means = 520), were randomly selected from the heads available at the National Breeding Center, Department of Livestock Development and from the herd of the Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University. Buffaloes were housed at the Veterinary Student Training Center at Nakorn Pathom province, and fed daily with roughage and water ad lib., together with the supplementation of 4 kg of concentrate containing 14% of protein per animal. This study was conducted on the same animals during March to June 2004 (hot season with average outdoor temperature of 31.7°C, range between 26.0°C and 35.1°C, and Thermal Humidity Index, THI = 84) determined as low breeding season and November 2004 to January 2005 (cool season with average outdoor temperature of 31.3°C, range between 22.7°C and 33.9°C, THI = 81) determined as high breeding season. In order to synchronize estrus, each buffalo was received 6 mg of a progesterone ear implant (Crestar®, Intervet, The Netherlands) for 10 days and a luteolytic dose (25 mg) of PGF2α (Lutalyse®, Pfizer, USA) at the time of implant removal. Ovulation was monitored during the 5 days following implant removal. Two thousands IU of hCG (Chorulon®, Intervet, The Netherlands) were administered at the end of the 5 days from implant removal in the event ovulation had not occurred.

Follicle development: Follicle development was recorded daily by per rectum and investigated by real time B-mode ultrasound unit equipped with 5 MHz transvaginal probe (Aloka, SSD-210 Tokyo, Japan), starting the day following prostaglandin administration and implant removal. Ultrasound monitoring was always performed by the same person, and follicles with a diameter ≥ 2 mm were recorded and visualized on a sheet map and classified into subordinated and dominant follicles. Heat detection was performed at least twice daily (AM/PM) by use of an epididymectomized bull. Follicular dynamics was monitored for at least 2 consecutive estrous cycles on each cycling buffalo.

Blood collection for hormonal profile: Blood samples (10 ml) from the jugular vein were collected daily from each animal starting the day following implant removal. Samples were stored into sterile plastic tubes without anticoagulant and subsequently centrifuged at 1,500g for 10 min. Serum was stored in Eppendorf tube at -200°C until ready for progesterone analysis. Progesterone monitoring was to confirm ovulation and to determine luteal function after ovulation.

Hormonal assay: Progesterone (P4) values were determined by RIA kit (Hegstag, 1992).

Statistical analysis: When a follicle diameter was recorded without increase in size for consecutive days, the first day was taken as the day of attained largest diameter described as dominant follicle. The duration of a dominant follicle within the ovary was defined as the interval of time (days) elapsed between its appearance and disappearance as a follicle ≥ 4 mm. Growth rate (mm/d) of each dominant follicle was calculated by the largest size attained divided by the number of days between its appearance as a follicle ≥ 4 mm and its maximum size. The following transformation of the data were made to normalize error distribution: 1) log for maximum size and duration of follicle, and 2) square root for growth rate. Differences between duration of estrous cycle, maximum size of dominant follicles, duration and growth rate of dominant follicles, as well as mean number of total available follicles were tested using t-test. Numerical values are expressed as mean ± SD throughout the text.

Results

Estrous cycles and follicle development

Nine complete estrous cycles were recorded from 4 buffaloes during the hot season and 13 estrous cycles from 5 buffaloes during the cool season. During summer months, 77.8% (7 buffaloes out of 9) of estrous cycles were characterized by 2-wave development of follicles whereas 22.2% (2 buffaloes out of 9) were recorded as 1-wave cycle. The length of estrous cycle was 22.6±1.9 days in a 2-wave cycle and 25.5±3.5 days in a 1-wave cycle (p<0.05). During winter months, 76.9% (10 buffaloes out of 13) of estrous cycles were characterized by 2-wave development of follicles whereas 23.1% (3 buffaloes out of 13) were recorded as 1-wave. The length of estrous cycle was 22.5±1.9 days in a 2-wave cycle and 20.7±2.8 in a 1-wave cycle (p>0.05). Overall, from the total number of recorded estrous cycles (n = 22), five (22.7%) were characterized by 1-wave of follicle development and seventeen (77.3%) by 2-wave of follicle development (p<0.05).

One-wave follicular dynamics

Main parameters referring to follicular dynamics of swamp buffaloes with estrous cycles characterized by one follicular wave during the hot and cool seasons are reported in Table 1. With ovulation time defined as day 0, waves emerged on day 2.3±0.5 and day 1.8±0.4 of the following cycle during the hot and cool season, respectively (p>0.05). Selection of the dominant follicle, characterized by the deviation in growth rate between the first and second largest follicles, occurred on day 7.6±1.8 and day 8.2±1.7 in summer and winter months, respectively (p>0.05). The largest diameter of first subordinated follicle was 8.7±1.7 vs 9.3±2.4 mm in hot and cool season, respectively (p>0.05). The largest size of the dominant follicle was recorded on day 13.5±1.2 and on day 12.6±1.5 during the hot and cool season, respectively (p>0.05). Likewise, the largest diameter recorded was 14.5±2.1 and 16.4±2.7 mm during the hot and cool season, respectively (p>0.05) (Figure 1).

Two-wave follicular dynamics

Main parameters of follicular dynamics in estrous cycle of swamp buffaloes characterized by two waves of follicle development during the hot and cool seasons, are reported in Table 2 and 3. During the hot season, the first wave emerged on day 1.2±0.3 of the cycle and was followed by selection of the dominant follicle on day 7.4±1.3. Growth of the dominant follicle continued until day 9.8±0.9, followed by beginning of regression on day 11.3±0.8. The second wave emerged on day 11.4±0.8, with the ovulatory follicle being selected on day 15.8±2.4, and reaching the largest diameter of 10.3±1.2 mm on day 19.7±1.1. During the cool season, the first wave emerged on day 0.9±0.4 of the cycle and was followed by selection of the dominant follicle on day 7.8±0.8. Development continued until day 10.6±1.3 followed by beginning of regression on day 12.4±1.6. The second wave emerged on day 10.7±0.9, with the ovulatory follicle being selected on day 15.4±1.6, and reaching its largest diameter of 12.8±1.2 mm on day 18.9±1.7. The dominant follicle of

Table 1 Follicle end points in swamp buffalos displaying one follicular wave during estrous cycles in the hot and cool season (mean ± SD).

Parameters	1-wave (hot season)	1-wave (cool season)	*p*-value
No. of estrous cycle (%)	2/9 (22.2%)	3/13 (23.1)	$p>0.05$
Wave onset (day)	2.3 ± 0.5	1.8 ± 0.4	$p>0.05$
Wave length (days)	25.5 ± 3.5	20.7 ± 2.8	$p>0.05$
Dominant follicle			
Maximum diameter (mm)	14.5 ± 2.1	16.4 ± 2.7	$p>0.05$
Day of maximum diameter	13.5 ± 1.2	12.6 ± 1.5	$p>0.05$
Growth rate (mm/day)	0.6 ± 0.3	0.7 ± 0.2	$p>0.05$
Day of deviation	7.6 ± 1.8	8.2 ± 1.7	$p>0.05$
Length of growth phase (days)	11.2 ± 0.8	10.8 ± 0.9	$p>0.05$
Largest subordinate follicle			
Maximum diameter (mm)	8.7 ± 1.7	9.3 ± 2.4	$p>0.05$
Growth rate (mm/day)	0.5 ± 0.1	0.6 ± 0.1	$p>0.05$

Table 2 Follicle end points in swamp buffalos displaying 2 follicular waves during estrous cycles in the hot season (mean ± SD).

Parameters	Follicular wave	
	first wave	second wave
No. of estrous cycle (%)	-	7/9 (78.8%)
Wave onset (day)	1.2 ± 0.3	11.4 ± 0.8
Wave length (days)	11.8 ± 2.3	10.6 ± 2.6
Dominant follicle		
Maximum diameter (mm)	10.9 ± 2.1	10.3 ± 1.2
Day of maximum diameter	9.8 ± 0.9	19.7 ± 1.1
Growth rate (mm/day)	0.7 ± 0.2	0.7 ± 0.1
Day of deviation	7.4 ± 1.3	15.8 ± 2.4
Length of growth phase (days)	8.4 ± 0.7	8.2 ± 1.3
Largest subordinate follicle		
Maximum diameter (mm)	7.7 ± 1.6	7.9 ± 1.3
Growth rate (mm/day)	0.6 ± 0.3	0.6 ± 0.2

Table 3 Follicle end points in swamp buffalos displaying 2 follicular waves during estrous cycles in the cool season (mean ± SD).

Parameters	Follicular wave	
	first wave	second wave
No. of estrous cycle (%)	-	10/13 (76.9)
Wave onset (day)	0.9 ± 0.4	10.7 ± 0.9
Wave length (days)	12.2 ± 2.1	10.4 ± 1.8
Dominant follicle		
Maximum diameter (mm)	8.7 ± 1.8	12.8 ± 1.2
Day of maximum diameter	10.6 ± 1.3	18.9 ± 1.7
Growth rate (mm/day)	0.7 ± 0.2	0.8 ± 0.1
Day of deviation	7.8 ± 0.8	15.4 ± 1.6
Length of growth phase (days)	9.5 ± 0.9	8.1 ± 1.3
Largest subordinate follicle		
Maximum diameter (mm)	8.1 ± 1.5	8.6 ± 1.4
Growth rate (mm/day)	0.6 ± 0.4	0.6 ± 0.3

Table 4 Progesterone values (ng/ml) during estrous cycle with 1-wave and 2-wave follicle development in swamp buffaloes during hot and cool seasons (mean ± SD).

	1-wave		2-wave		
	Hot	Cool	Hot	Cool	Mean
Estrus	0.11 ± 0.08	0.13 ± 0.05	0.04 ±0.07	0.09±0.04	0.07±0.03
Mid luteal phase	2.25 ± 0.7	2.06 ± 0.3	2.31 ±0.4	2.54±0.5	2.37±0.5
Late luteal phase	1.03 ± 0.4	1.14 ± 0.6	0.84 ±0.5	1.10±0.3	0.98±0.2

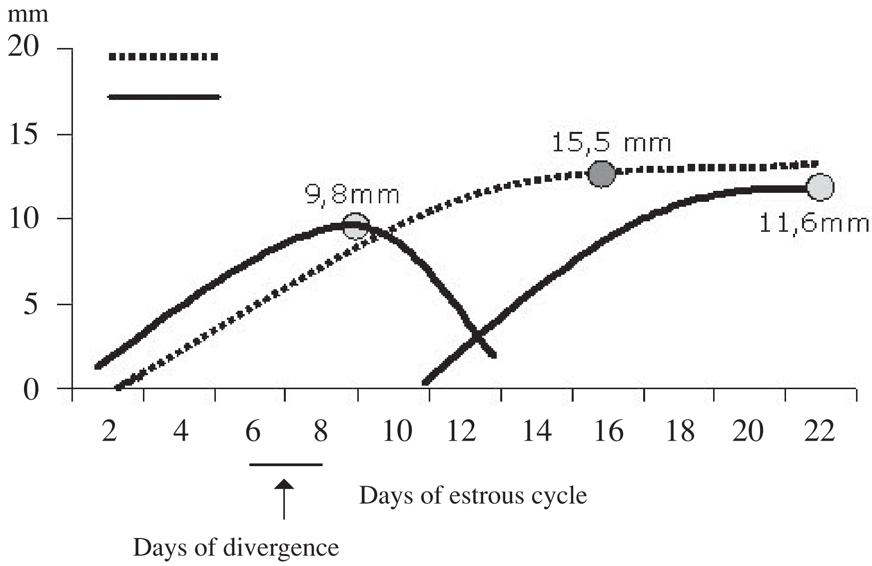

Figure 1. Follicular development in swamp buffalo

the first wave was recorded more frequently to the right rather than the left ovary (68.7% vs 31.3%; $p<0.05$), and the same trend was recorded for the second dominant and ovulating follicle with a frequency of 62.3% vs 37.7% ($p<0.05$), respectively. The overall higher incidence of ovulated dominant follicles on the right ovary of all the monitored follicular waves was paralleled by the higher frequency (64.5%) of corpora lutea recorded on the same ovary (Figure 1).

Progesterone values

Progesterone values followed a similar trend for both 1- and 2-wave cycles during the hot and cool season. No significant differences were recorded between seasons and at targeted time during the estrous cycle for both kind of wave pattern. Therefore, pooling data for both 1- and 2-wave cycles and seasons, mean progesterone value at estrus and ovulation was 0.07±0.03 ng/ml, but after ovulation, the mean progesterone increased to 2.37±0.5 ng/ml at the mid luteal phase and later, decreased at the late luteal phase was 0.98±0.2 ng/ml. Furthermore, progesterone profiles clearly gave indications that one buffalo was cyclic without apparent signs of estrus. Detailed progesterone values are given in Table 4.

Discussion

The need to apply the reproductive technology for improving production and reproductive traits of swamp buffaloes has urged researchers to investigate further on basic aspects of their ovarian physiology. Within this framework, a comparison of follicular turnover in swamp buffaloes between low breeding (hot) and high breeding (cool) seasons has been carried out for the first time in this study. Swamp buffaloes are characterized by a number of physiological features which are mostly similar to those already reported in riverine buffaloes. They suffer from a reproductive function which is heavily affected by the environment, nutrition level and age (Zicarelli, 1997). Ultimately, reproductive efficiency can be related to the fine tuning of ovarian function, the down-stream reproductive organs and hormonal balance. This is why the study of ovarian follicular dynamics has played a role of paramount importance for the understanding, improvement and control of reproductive function in livestock animals (Lucy et al., 1992).

Swamp buffaloes, like their riverine counterpart, are tangentially seasonal breeders (Esposito et al., 1992; Dobson and Kamonpatana, 1986). Therefore their reproductive cycles and follicle development within cycles can be expected to be altered by the unfavorable season leading possibly to a reduced reproductive efficiency. The most disruptive of such deviations from a cyclic pattern of follicle development is the anestrus condition. In riverine buffaloes seasonal anestrus has been differentiated into a deeper and a more temporary or superficial condition (Zicarelli, 1997). In fact, deep anestrus is characterized by a very slow or almost absent follicle turnover which can possibly be resolved only by the exogenous administration of progesterone implant together with gonadotrophin supplementation in order to stimulate ovarian function. On the contrary, a milder degree of anestrus condition, characterized nevertheless by a clear pattern of follicle turnover and presence of large size follicles, can be overcome by the usual treatment with progesterone implant without the need of gonadotrophin supplementation (Presicce et al., 2004). It is clear then, how instrumental can be the understanding of follicular turnover in swamp buffaloes under different seasonal conditions for breeding purposes. The presence of one follicular wave pattern in swamp buffalo in 22% of estrus cycles may suggest the presence of a dominant follicle that persist for more then 10-14 days. Since persistent follicles usually produce oocytes of low quality that are difficult to be fertilized, this could be one of the cause of intrinsic low fertility in swamp buffaloes. Moreover, the presence of one follicular wave may generate a more variable duration of estrus and make prediction of ovulation and identification of the best time for AI more tedious. Therefore, hormonal treatments that reduce the presence of persistent follicles in swamp buffaloes may be useful approaches to increase infertility.

From previous studies in riverine buffaloes, it appears that 2-wave cycles of follicle development are predominant over 1-wave and 3-wave cycles (Taneja et al., 1996). In this study the same trend was confirmed in both the hot and cool season. In comparison with similar studies performed by Taneja et al. (1996) and by Presicce et al. (2004) in riverine buffaloes displaying a 2-wave cycle, in this study a reduced largest size of 1st and 2nd wave dominant follicles as well as a reduced growth rate of dominant follicles was reported. Although a direct comparison between riverine and swamp type buffaloes has never been carried out, it can be speculated that such differences, albeit not statistically measurable and under the conditions imposed by the studies considered, could be speculatively ascribed to a different genetic makeup of the two subspecies. On the contrary, in a typical 2-wave cycle the expected time of follicle deviation leading to dominance over the second largest and other subordinate follicles found in this study, is in accordance with previous reports by Taneja et al. (1996) and Presicce et al. (2004). Furthermore, in this study growth rate of dominant follicles between 1-wave vs 2-wave cycles has been found similar. Largest attained diameter of dominant follicle in 1-wave cycle in both hot and cool season though, was reported to be larger than the ovulating dominant follicle in a 2-wave cycle. It is known that the fate of the dominant follicle associated to the first wave of follicle development is dependent on CL function and consequently on the level of blood progesterone. Progesterone levels at mid-luteal phase are usually sufficient to reduce LH frequency, determine regression of the first dominant follicle and initiate a new wave of follicle development (Lucy et al., 1992). In this study, recorded larger size of the ovulating dominant follicle in 1-wave cycles when compared to 2-wave cycles, could be ascribed to a longer exposure to LH frequency before its reduction in conjunction to progesterone rise. One-wave cycles of follicle development have been recently reported as normally occurring in suckled buffaloes, and an atypical growth of the dominant follicle has been described as characterized by an initial growth, a regression and a final re-growth followed by ovulation (Awasthi et al., 2006). In accordance with previous studies (Pathak, 1992; Jainudeen and Hafez, 1993), estrous behavior was mostly recorded in the cooler hours of morning and evening, confirming the notion that mating behavior in buffaloes usually ceases during daylight hours (Tailor et al., 1990). In this study a epididymectomized bull was used to detect animals in estrus following synchronization protocol and the corresponding behavior was not found to be significantly affected by the season, although signs of estrus were probably more pronounced during the nights of the cool season. The response of swamp buffaloes to the protocol of synchronization of estrus was not successful in both seasons. As a consequence, all but one buffalo had to be subjected to exogenous hCG administration at the end of the first five days following implant removal. A higher number of ovulations were consequently recorded on a total of nine buffaloes between both seasons. The difficulty in inducing ovulation in swamp buffaloes following a progesterone based synchronization protocol may be speculatively related to an insufficient endogenous LH availability. It is possible that the time given to build up LH reserve in the course of some progesterone based protocols for synchronization of estrus, may not be adequate or else the concentration of circulating progesterone when using Crestar® for a limited amount of time may not reach the critical threshold necessary to unleash LH release following progesterone device removal. Unsuccessful synchronization for estrus and timely ovulation using a progesterone based protocol has also been reported by Presicce et al. (2004) in riverine heifer buffaloes.

The length of the estrous cycle in buffaloes are generally accepted as more variable than those observed in cattle (Bhattacharya, 1974). Cycles ranging between 18 to 26 days are considered normal in buffaloes (Kanai and Shimizu, 1983; Singh et al., 1984) and length of 1-wave and 2-wave cycles in this study fall therefore within the previously reported range (Presicce et al., 2004; Taneja et al., 1996; Singh et al., 1984). Within 1-wave and 2-wave cycles, no significant differences among the various parameters of follicular turnover under scrutiny have been highlighted in this study between seasons. A possible explanation for such findings can be tracked down on similar climatic conditions that have characterized the two seasons part of this study, in addition to a similar well balanced diet and energy intake given to animals in both seasons. The absence of any real dramatic climatic change between seasons and similar good feeding regimen must have obscured any possible appreciable differences among follicle end points and the cycle as a whole.

In accordance with previous reports by Dobson and Kamonpatana (1986), progesterone values at estrus in both seasons and for both 1-wave and 2-wave cycles were recorded at baseline values and did not rise until day 5 of the following estrous cycle. Overall, in this study the mean progesterone level in cyclic animals at estrus was 0.07±0.03 ng/ml with an increase between day 4 and 7, reaching a peak around day 15 and reduced nearly to baseline levels at approximately day 18 of the estrous cycle. The cyclic pattern of progesterone concentration observed in this study was in line with the changes found in functional CL in the course of the estrous cycle. Between day 16 and 21 of estrous cycle, the expected process of luteolysis resulted in a precipitous fall in progesterone to a basal concentration, as already reported by Chau et al. (1983). Progesterone profile was helpful in highlighting ovarian cyclic condition in one buffalo not showing any signs of estrus, that otherwise would have come unnoticed, in agreement with previous reports of silent estrus in riverine buffaloes (Zicarelli, 1997).

In conclusion, results from this study confirm the typical pattern of follicle wave development in swamp buffalo.

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