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Full Length Article

Puberty, Ovarian Cycle, Ovulation and Post-Partum Uterus Recovery in River and Swamp Type Crossbred Buffaloes

Adili Abulaiti[1], Guohua Hua[1,2], Hafiz Ishfaq Ahmad[1] and Liguo Yang[1,2*]
[1*]*Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Ministry of China, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China*
[2]*Hubei Province's Engineering Research Center in Buffalo Breeding and Products, Wuhan, 430070, People's Republic of China*
[*]For correspondence: yangliguo2006@qq.com

Abstract

This study was designed to determine the maturity of Chinese crossbred buffalos as evidenced by different parameters. For this purpose, a total of 32 crossbred buffalo heifers at an average 16 months of ages with average body weight 245.0 ± 11.5 kg were used to study the follicular development by ultrasonography until first ovulation. Results revealed that the age of puberty (20~24 months) was related to the diameter of ovulating dominant follicle with following measurements of different parameters in crossbred buffalo heifers: maximum diameter of ovulating dominant follicle 10.2 ± 1.0 mm, body weight 298.3 ± 23.2 kg, height 119.1 ± 3.4 cm, body length 119.4 ± 5.1 cm, chest girth 164.2 ± 3.8 cm, and abdominal girth 187.0 ± 4.1 cm. The average puberty time for Riverine x Swamp type crossbred buffalo was 23.59 ± 0.4 months. The ultrasonographic examination revealed that in healthy crossbred buffalo cervix, gravid and un-gravid uterine horn recovery was 33.6 ± 2.4, 36.8 ± 3.4 and 31.6 ± 1.8 days; however, in uterine diseased crossbred buffalo population, the recovery days were 43.1 ± 5.2, 45.6 ± 6.8, and 39.6 ± 5.7 days, respectively. Ovulation time for first, second and third estrus cycle in healthy post-partum buffalo was 27.7 ± 7.1, 48.5 ± 8.4, and 65.0 ± 13.8 days, respectively. These parameters could be reliable indicators for accurate artificial insemination programs, super-ovulation and embryo transfer technology. So, that breeding can provide authentic theoretical basis for research to improve the fertility and to attain more benefits from crossbred buffalo farming. © 2018 Friends Science Publishers

Keywords: Ultrasonography; Heifers; Puberty; Post-partum; Ovarian follicular dynamics; Crossbred buffalo

Introduction

Buffalo is an important livestock resource in Asia; however its reproduction is constrained due to biological and management problems. The better understanding of postpartum buffalo uterine recover, follicle development rules, and fix time artificial insemination are essential for the improvement of the reproductive performance in crossbred buffaloes. Swamp type buffalo have lower milk production (98.3 million ton/year) as compared to riverine buffalo (Lu *et al.,* 2015). Crossbreeding of swamp with riverine buffalo has been proven to be an efficient strategy for improving milk production of local swamp buffalo (Khajarern and Khajarern, 1991). As the age at puberty in buffalo has been found to be highly inherited that`s why, the description of puberty and early sexual maturity is an important tool for selection within the males of a particular breed. Therefore, choosing precocious heifers might be an efficient mean of decreasing the age at puberty in these animals and this access is being accepted in marketable practice (Campanile *et al.*, 2009; Khalifa *et al.*, 2013).

Several factors such as breed, nutrition, genotype, climate, strain differences, management, and the social environment, season of birth and health conditions affect the onset of puberty. Similarly, energy deficiency in the body delays puberty and reduce animal fertility, body weight gain, and other productive parameters (Silva *et al.*, 2010). Researchers agreed that early pubertal age is associated with the time of birth and the nutritional planes (Khalifa *et al.*, 2013; Perry, 2016), Buffalo heifers typically gain puberty when they arrive at approximately 55–60% of their adult body weight. However, the age at which they reach at puberty can be extremely variable, ranging from 18 to 46 months (Jainudeen and Hafez, 2016). Various studies from several countries (Medvedev *et al.*, 1980) demonstrated that under convenient conditions riverine buffalo exhibits first estrus at 15–18 months of age, while the swamp at 21–24 months. The body weight at puberty, which is around 200–300 kg for the swamp type and 250–400 kg for the river type buffalo is strongly affected by the genotype (Naz and Ahmad,

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2006). While, buffalo attains puberty later than cattle, however buffalo has a longer reproductive life, which tends to compensate for this economic weakness at an average age of 31.53 ± 0.88 months with a body weight of 380.67 ± 6.42 kg (Haldar and Prakash, 2006). Moreover, the onset of puberty is different in the similar type animals depending upon various factors. North China swamp type heifer has started its ovulation period at 11–15 months of age, while the Yak of the Qinghai-Tibet plateau has first ovulation between the 2.5–3.5 years of age (Sabia *et al.,* 2014; Gallegocalvo *et al.,* 2015). The younger buffalo that attain early puberty and maturity can breed timely, receive early pregnancy and produce premature calves. Sexually matured buffalo heifers have been found enhancing the ovarian circumference with age, which is more pronounced around 13 months old (Naz and Ahmad, 2006).

In buffalo, postpartum reproductive function recovery can affect the milk production, fertility, and lifelong milk yield, ultimately affect the production efficiency, postpartum estrus, ovulation and the next pregnancy (Crowe and Mullen, 2013). The first postpartum estrus in swamp, river and lakeside buffalo are around 55 (26 ~ 116 days) 42 and 63 days, respectively. Aging and malnutrition delay postpartum estrus 7 to 147 days. Calving season and other factors also affect the first postpartum estrus time (Yindee *et al.,* 2007). Crossbred buffalo postpartum and pregnant period are closely related to the buffalo postpartum uterine recovery time. There habilitation of uterus and ovarian function is necessary for further fertility as well as reduced interval in reproduction (Ramírez *et al.,* 2009).

Although rivers and swamp buffalo follicle development pattern research has been reported, but these two types of buffalo chromosome number is different, River water buffalo 2n=50, swamp buffalo 2n=48, hybrids containing 2n=49 chromosomes it remains need further research. This study was conducted to investigate the follicle growth rules for estrus induction and detection, ovulation control, super ovulation, embryo transfer and improved artificial insemination, which seems essential for improving crossbred buffalo fertility and distribution of genetic material in enhancing fertility rates in buffalo industry.

Materials and Methods

Experimental Animals

A total of 49 crossbred Chinese buffalo (Murrah X Nili Ravi X Chinese water buffalo) (puberty, n=32; postpartum, n=17) belonging to Hubei cattle breeding base of Animal Husbandry Co. Pvt. Ltd. Jingmen, Shayang County, Hubei Province, China, were used for experiment during May 2014 to June 2015. Experimental animals were selected based on sound health, physically good condition, disease free and having normal estrus cycle and body condition score (BCS). Experimental animals were divided into two groups, postpartum stage (n=17), puberty stage (n=32). Animals were adlib feeding with a total mixed ration (TMR) machine immersion corn silage and dry straw, supplemented by leguminous and non-leguminous green forage and concentrate. Ages of experimental animals' were 4–8 years for postpartum stage group and 17–21 months for puberty stage group. All buffalo were between the second and the fourth parity and were free from reproductive disorders. They were continued under similar management conditions and stall-feeding with water adlib. The buffalo were machine-milked twice a day and limited suckling of a calf preceded each milking.

The mean body weights for postpartum and puberty stages of experimental buffalo were 588.1 ± 89.5 kg and 298.3 ± 23.2 kg, respectively, while the mean chest girths were 212.0 ± 11.7 cm and 166.8 ± 7.8 cm, respectively. Moreover, the mean body height, body length and abdominal girth for the same experimental animals of postpartum (132.3 ± 4.9 cm, 128.1 ± 5.1 cm, 120.3 ± 4.7 cm) and puberty (243.4 ± 15.2 cm, 211.0 ± 13.2 cm, 188.9 ± 17.4 cm) were recorded. In this study, we applied ultrasound technology to examine hybridization in pubertal and puberty onset time to attain the objectives of improved fertility and milk production previously described (Neglia *et al.,* 2014). Ovarian follicular dynamics were monitored twice a daily at 9:00 a.m. and 2:00 p.m. with ultrasound equipment desktop B-type veterinary ultrasound scanner, WED - 9618 - v, equipped with LV2-3/6.5 MHz rectal probes.

Follicle Development Detection

Ultrasound monitored of the ovarian follicles was conducted on a daily basis starting from Day 0 of the experiment to the day of ovulation and the follicle development was monitored twice a daily at 9:00 a.m. and 2:00 p.m. using a real time ultrasound scanner (Desktop B-type veterinary ultrasound scanner, WED - 9618 - V, equipped with LV2-3/6.5 MHz rectal probe). A follicle of 10 mm in diameter was considered to be a dominant follicle morphologically. Each examination was carried out by the same operator to reduce measurement error. A dominant follicle and its cohort were defined as a follicular wave (Fortune, 1993). In this study, the day of onset follicular wave was observed as the day when the dominant follicle was first discovered more than 4 mm in diameter. For ovarian follicle diameter, changes were checked every four hours until the end of estrus.

Statistical Analysis

The data were measured as mean ± SE and subjected to analysis of variance to study the effect of three factors (two physiology herd, different follicle wave, left and right ovary). Statistical analyses were carried out using one way analysis of variance (ANOVA) to analyze the

data using Statistical Package for the Social Sciences (SPSS 8.0).

Results

Onset of Puberty in (riverine x swamp) Crossbred Buffalo

Results showed that the average age at puberty for (riverine x swamp) crossbred buffalo was 22.93 ± 1.6 months, onset of puberty average age and 23~24 month age no significance difference (P>0.05). Out of 32 buffalo heifers, large numbers (n=27) have onset of puberty at 20~24 months; whereas, the average age of puberty was 22.93 ± 1.6 months. Mean cervix diameter was 20.4 ± 1.5 mm, left and right uterine horn were 19.0 ± 1.5 mm and 19.0 ± 2.0 mm respectively. Likewise, average follicle diameter of left and right ovaries at age of puberty was 10.1 ± 1.1 mm and 10.2 ± 1.0 mm, respectively. At puberty the average body weight, height, body length, chest girth and abdominal girth were 298.3 ± 23.2 kg, 119.1 ± 3.4 cm, 119.4 ± 5.1 cm,164.2 ± 3.8 cm, and 186.9 ± 4.1 cm, respectively, body weight and height found has a significance difference between 20~22 month and 23~24 month (Table 1).

Based on genetic and environmental factors, the results demonstrated that the onset of puberty was characterized by the first ovulation time and maximum DF diameter follicle diameter, which happened between 20 and 24 months of age. Sexual maturation of buffalo involved an increase in ovarian circumferences with the age that was more obvious around 20 months old. The rate of follicular growth stayed the same throughout the age vary but the growing phase lasted longer (Fig. 1).

The Results showed that the cervix and uterine horn growth rate in before puberty and after puberty of (riverine x swamp) crossbred buffalo have non-significant difference for all data, the result explain that onset of puberty uterine development is not perfect, not ready for artificial insemination (Table 2).

Uterine Parturition Time and Follicle Developing in (riverine x swamp) Crossbred Buffalo

Results showed that the uterine recovery with postpartum estrus and ovulation has a close relationship. The above mentioned table showed the first, second and third ovulation time from left and right ovary in post-partum crossbred buffalos. Most of the post-partum buffalos were observed with two wave estrus cycle. After parturition first, second and third average ovulation time were 27.7 ± 7.1, 48.5 ± 8.4 and 65 ± 13.8 days, respectively (Table 3).

The DF onset time was explained in Table 4 with follicle wave duration and largest follicle appearance time in gravid and un-gravid horn in post-partum buffalos for two follicular wave cycle during the estrus cycle. Dominant follicle onset time was found higher in gravid horn (1.5 ± 0.9

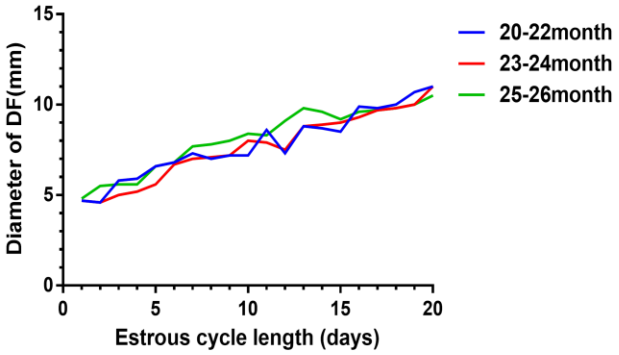

Fig. 1: Follicle growth principles of puberty stage of (riverine x swamp) type crossbred buffalos at 20~22 months (n=12), 23~24 months (n=15), 25~26 months (n=5) of age

days). First wave duration time was found highest in un-gravid horn (12.5 ± 2.9 days). Maximum diameter of DF was found in un-gravid horn follicle (9.5 ± 2.1 mm). Moreover, the ovulation time was highest in un-gravid horn (10.6 ± 2.1 days). In two-wave cycle second wave DF onset time was found highest in un-gravid horn (13.4 ± 2.7 days), whereas the longest wave duration was found in gravid horn (10.9 ± 2.9 days). The greatest ovulation time and maximum size of DF were observed in gravid horn, whereas estrus cycle duration and maximum size were 18.8 ± 2.3 days and 11.6 ± 2.5 mm, respectively. In two-wave cycle the greatest DF onset time, duration of estrus cycle, maximum size DF and ovulation time was observed in un-gravid horn (Table 4).

Inactive ovaries are one of the most important reasons for limiting the estrus behavior after post-partum. First wave DF onset time was highest in inactive follicle (2.3 ± 1.0 days). In (riverine x swamp) crossbred buffalo, wave continuous time and DF onset time, ovulation time and maximum size follicle, all data were found with no significant difference (P<0.05) in active and inactive follicle for two wave cycle of estrus buffalo. In two-wave cycle, ovulation time and maximum size follicle were found with significant difference between active and inactive growing follicle (P<0.05). The results explained that post-partum herd two-wave follicle estrus cycle had left ovary follicles, where active follicles were 35.29% (6/17) and inactive follicles were 64.71% (11/17), whereas, for right ovary follicles active follicles were 64.29% (11/17) and inactive follicle were 35.29% (6/17), Crossbred buffalo left side horn pregnancy rate 7/17 (41.2%), right side horn pregnancy rate was 58.8% (10/17), result explain that most of time artificial insemination in right side ovary (Table 4).

Results for uterine cervix recovery, gravid uterine horn and un-gravid uterine horn in healthy and uterine diseased (riverine x swamp) crossbred buffalo after parturition has been shown (Fig. 2). Significant difference (P<0.05) was observed for all cervix, gravid uterine horn and un-gravid uterine horn in healthy and uterine diseased crossbred buffalos (Fig. 3). The greatest

Table 1: Onset of puberty and Body measurements at puberty stage of (riverine x swamp) type crossbred buffalos

Body measurement	Average (n=32)	20-22 month (n=12)	23-24 month (n=15)	25-26 month (n=5)
Weight (kg)	298.3 ± 23.2ab	276.0 ± 16.76a	320.67 ± 48.20b	311.50 ± 19.07ab
Onset of puberty (month)	22.9 ± 1.6a	21.27 ± 0.6b	23.59 ± 0.4a	25.45 ± 0.52c
Height (cm)	119.1 ± 3.4ab	116.38 ± 2.52a	122.27 ± 4.71b	121.0 ± 1.73ab
Body length (cm)	119.4 ± 5.1a	117.29 ± 5.42a	122.20 ± 6.59a	122.13 ± 5.44a
Chest girth (cm)	164.2 ± 3.8a	163.75 ± 4.49a	168.13 ± 10.19a	162.75 ± 4.57a
Abdominal girth (cm)	187.0 ± 4.1	188.46 ± 8.77a	188.10 ± 21.90a	182.50 ± 5.80a
Cervix (mm)	20.9 ± 3.3a	19.75 ± 1.91a	22.07 ± 4.17a	20.25 ± 1.26a
Left side uterine horn (mm)	19.3 ± 4.1a	18.0 ± 2.59a	20.47 ± 5.26a	18.75 ± 1.5a
Left side follicle (mm)	10.1 ± 1.1a	10.2 ± 0.9a	10.1 ± 0.9a	10.8 ± 1.3a
Right side uterine horn (mm)	19.4 ± 3.8a	17.58 ± 2.64a	20.73 ± 4.54a	19.75 ± 0.96a
Right side follicle (mm)	10.2 ± 1.0a	9.9 ± 1.1a	10.3 ± 0.9a	10.2 ± 1.0a

Mean ± standard deviation. a, b indicate difference between in the rows. Values sharing same letters differ non-significantly (P>0.05)

Table 2: Uterine cervix and horn growth rate at before and after puberty stages (riverine x swamp) type crossbred buffalos

Physiological stages of Buffalos	The cervix growth rate (mm/d)	R2	Right uterine horn growth rate (mm/d)	R2	Left uterine horn growth rate (mm/d)	R2
Before puberty (n=25)	0.0108a	0.9296	0.0109a	0.874	0.0153a	0.9479
After puberty (n=20)	0.0129a	0.941	0.0137a	0.8944	0.0107a	0.8533

Mean ± standard deviation. a, b indicate difference between in the columns. Values sharing same letters differ non-significantly (P>0.05)

Table 3: Ovulation time for first, second and third estrus cycles in post-partum buffalos in two-wave estrus cycle

Post-Partum buffalos	First ovulation time (Days)	Second ovulation time (Days)	Third ovulation time (Days)
Left side ovary (n=10)	23.8 ± 6.5a	45.5 ± 9.4a	61.3 ± 16.2a
Right side ovary (n=4)	31.6 ± 5.7a	51.6 ± 6.9a	68.8 ± 12.1a
Average (n=14)	27.7 ± 7.1a	48.5 ± 8.4a	65.0 ± 13.8a

Mean ± standard deviation. a, b indicate difference between in the columns. Values sharing same letters differ non-significantly (P>0.05)

Table 4: DF onset time, follicle wave duration and largest follicle appear time in gravid and un-gravid horn , active and inactive follicles in post-partum buffalos for two follicular wave cycles**,** Incidence of gravid and un-gravid horn ,active or inactive DF in left and right side ovary

Post-Partum buffalo (n=14)	Follicle dynamics	gravid horn (n=11)	Un-gravid horn (n=10)	Active follicle (n=9)	Inactive Follicle (n=5)
First follicle wave	DF appear time	1.2 ± 1.1[a]	1.0 ± 1.5[a]	1.3 ± 1.7a	2.3 ± 1.1b
	Duration	11.1 ± 2.9[a]	12.5 ± 2.9[a]	12.3 ± 2.9a	11.1 ± 2.8a
	Maximal diameter of DF	9.2 ± 1.9[a]	9.5 ± 2.1[a]	9.5 ± 2.2a	9.3 ± 1.8a
	largest follicle appear time	7.0 ± 2.0[a]	9.2 ± 2.3[a]	8.0 ± 2.1a	8.4 ± 2.9a
	Days from calvin（0day）	35.0 ± 10.2[a]	29.1 ± 6.2[a]	29.0 ± 9.4[a]	35.3 ± 12.2[a]
Second follicle wave	DF appear time	12.4 ± 2.3[a]	13.4 ± 2.7[a]	12.3 ± 1.6a	12.2 ± 1.1a
	Duration	10.9 ± 2.9[a]	10.2 ± 2.2[a]	10.0 ± 2.4a	10.7 ± 2.6a
	Maximal diameter of DF	10.2 ± 2.6[a]	11.6 ± 2.5[a]	12.1 ± 2.3a	9.8 ± 2.5b
	largest follicle appear time	17.1 ± 2.8[a]	18.8 ± 2.3[a]	20.1 ± 2.6a	16.9 ± 2.5b
	Days from Post-Partum	45.5 ± 8.2[a]	39.4 ± 7.5[a]	43.6 ± 8.5[a]	46.0 ± 12.6[a]
Percentage (%)	Left side ovary(n=4)	37.5%	62.5%	(35.71%)	(64.29%)
	Right side ovary(n=10)	62.5%	37.5%	(64.29%)	(35.71%)

Mean ± standard deviation. a, b indicate difference between gravid and un-gravid horn , active and inactive follicles in the rows. Values sharing same letters differ non-significantly (P>0.05)

uterine disease (n=7) buffalo cervix recovery time, gravid uterine horn and un-gravid uterine horn were 43.1 ± 5.2, 45.6 ± 6.8 and 39.6 ± 5.7 days, respectively (Fig. 3).

Normal buffalo (n=10) recovery time for cervix, gravid uterine horn and un-gravid uterine horn was 33.6 ± 2.4, 36.8 ± 3.4 and 31.6 ± 1.8 days, respectively (Fig. 3). Recovery time for cervix, gravid uterine horn and un-gravid uterine horn had a highly significant difference (P<0.05) between normal buffalo and uterine disease buffalo. Normal buffalo cervix, gravid horn and un-gravid horn recovery time were shorter than uterine diseased buffalo have a significant difference (P <0.05) (Fig. 3).

Discussion

The onset of puberty is an important factor for reproductive efficiency in farm animals. To date, several techniques have been utilized to advance puberty in farm

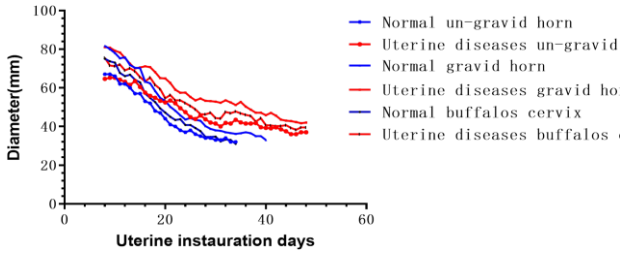

Fig. 2: Comparison between normal buffalo (n=10) and uterine diseases buffalo (n=7) cervix, gravid horn and un-gravid horn instauration days

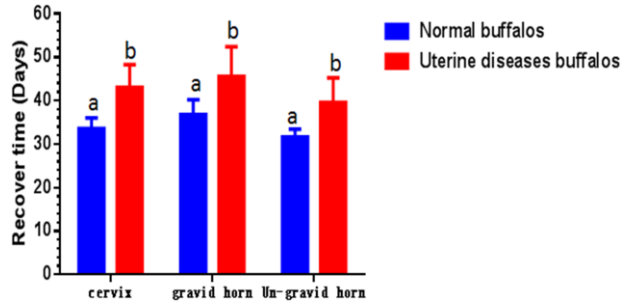

Fig. 3: Recover time of healthy and uterine diseased post-partum crossbred buffalos Mean ± standard deviation. a, b indicate difference between in the two group. Values sharing same letters differ non-significantly (P>0.05)

animals, we reported a first study describing the onset of puberty in Chinese (riverine x swamp) type crossbred buffalo at 22.93 ± 1.6 month age, body weight 298.3±23.2 kg (Table 1), which is close to previous findings in swamp buffalo (Naz and Ahmad, 2006). Chinese crossbred buffalo onset of puberty uterine development is not perfect, not ready for artificial insemination we suggest that buffalo heifers artificial insemination age ≥25 month is better. Nevertheless, there is limited information on advancing the puberty in dairy crossbred buffalo. We therefore, studied the effect of age on puberty advancement and circulating concentrations of body weight in late maturing crossbred buffalo (Madan and Prakash, 2007). The onset of puberty is characterized by the first ovulation (Rawlings *et al*., 2003), which occurs between 6 to 24 months of age depending on the genetic and environmental factors (Moran *et al.,* 1989). Sexual maturation of buffalo heifers involves an increase in ovarian circumferences with age that is more pronounced around 13 months of age (Carvalho *et al.*, 2013). Previous experiments determined that 32% of buffalos heifers became pregnant at 21 months of age and were estimated to have demonstrated early puberty (De Lucia *et al.,* 2002).

Previous studies described that an increasing protein intake resulted reduction in age and improving weight at puberty (Nogueira, 2004). Nutrition level can affect the age of sexual maturity in buffalo as well as the life-time reproductive performance and has significant influence on mature weight gain (Sekhon *et al.,* 1996). This study established that most of the buffalo under investigation have two follicular waves, with first and second follicular wave appearance time (1.2 ± 1.1 days), (12.4 ± 2.3 days) respectively. Our results are parallel with the previous study conducted in riverine buffalo or swamp buffalo (Kafi *et al.,* 2009). The onset of puberty of crossbred buffalo is related to first ovulation time and first ovulation dominant follicle diameter. The follicle diameter was 10.2 ± 1.0 mm at the onset of puberty. The onset of puberty is related to numerous factors, such as breed, age, season, body condition and production factors, growth and development of buffalo that mainly depend on the supply of nutrients, buffalo varieties, the quality of breast milk feeding, growth environment, management (Gallegocalvo *et al.,* 2015; Perry, 2016). Different studies revealed postpartum ovarian activity and the effect of subclinical uterine contagion on uterine participation and estrus cycle in buffalo, which providing our findings (Usmani *et al.,* 2001). Young buffalos take 25 to 32 days to bring its cervix and uterus to the normal position after their first parturition. It was described that 26 days were the average time interval for uterine recovery of milking water buffalo (Wang *et al.,* 2014). In Nili-Ravi buffalos the average interval from calving to completely recovery of the uterus and cervix were 25.6 ± 1.0 days (Shah, 2010). However, it was reported that in India and Sri Lankan buffalos the uterine recovery time is longer, that are 39 days and 42 days, respectively (Gill *et al.,* 1973). Although in the north China, Holstein cattle postpartum reproductive function recovery was studied (Wang *et al.,* 2014; Jeong *et al.,* 2015), but due to the vast area of country, the north-south differences in climate and the buffalo milk production, reproduction characteristics, etc. are highly variable. Therefore, it is necessary to differentiate the reproductive characteristics of buffalo in southern China from other regions (Benaich *et al.,* 1999).

However it has been reported that birth weight has significant effect on postpartum uterine involution (Cubillán *et al.,* 1999), though, there were reports that showed that prenatal and postnatal nutrition level (Usmani *et al.,* 1990), the body condition, rating of calf-birth and weight to the buffalo postpartum uterine involution period, were not significantly affected (Abdulatif and Hegazy, 1994). Previous studies revealed that calving in different months and seasons resulted in different, uterine recovery time (Perera, 2008). Generally considered, uterine recovery is slower in winter and faster in spring and summer, and claimed that the buffalo calving in summer has uterine recovery in only 15 days (Bahga and Gangwar, 1988), which is faster than in other seasons (Chauhan *et al.,* 1977). In dairy buffalos the mean interval of first ovulation was significantly (P<0.05) shorter in animals with moderate body conditions (47 days) at calving than in thin animals (63 days), but the interval of first open estrus (75 and 74 days, respectively) was not different (Barman *et al.,* 2011)). In swamp buffalo,

ovarian activity was resumed within 90 days in animals with a average BCS (Body Condition Scores) of 3.3 while those with BCS of 2.8 continued to be acyclic (Barman *et al.*, 2011). Research about uterine recovery in riverine buffalo and in swamp buffalo has been performed in China and as well as in many other parts of world, however this character has not been well studied yet in their crossbred. In present study, we studied riverine and swamp buffalo hybrid off springs. In normal buffalo (n=17), recovery time for cervix, gravid horn and un-gravid horn was 33.6 ± 2.4, 36.8 ± 3.4 and 31.6 ± 1.8 days, respectively (Fig. 3). Three independent studies (El-Wishy, 2007a) reported three different time (28 ± 6, 33 ± 3 and 33 ± 4 days, respectively) for uterine recovery of swamp buffalo. The results from our study are consistent with most of the previous studies.

In present study it was found that after a short period of time (< 25 d) the first ovulation occurred at the empty angle of ovary recorded about 64.71% (11/17), while for second time the ovulation occurred from ipsilateral ovary at the empty angle, which was accounted for 58.82% (10/17). Interestingly, the first ovulation occurred from the right side ovary (Perera, 2011). Postpartum body weight significantly ($P < 0.01$) effect the length of the acyclic period, as buffalos weighing >400 kg ovulated earlier (104 days) than those with weight ≤ 400 kg (206 days) (Kutluca *et al.*, 2006; Lesnoff and Lancelot, 2010). In another study no significant effect was mentioned (El-wishy, 2007b). Recovery of postpartum ovarian activity with quite late first estrus is the main reason of long calving interval in buffalo (Baruselli *et al.*, 2010). Post-partum buffalo after parturition first, second and third ovulation time were 27.7 ± 7.1,48.5 ± 8.4 and 65 ± 13.9 days, respectively, postpartum buffalo first ovulation time 27.7 ± 7.1 days, but uterine recover time need 33.6 ± 2.4 days, first time ovulation postpartum buffalo uterus has not fully recovered and not ready for artificial insemination, therefore we may suppose that postpartum buffalo artificial insemination time at second time ovulation 48.5 ± 8.4 days is good for increase pregnancy rate, Postpartum ovarian activity recovery time and late estrus high proportion is one of the causes of long calving interval time in buffalo (Gordon and Gordon, 2004). The present study demonstrated the first time of follicle dynamics of puberty in crossbred buffalos through ultrasonography evaluation. These techniques resulted in follicular wave emergence at consistently predictable manner followed by very tight synchronization of ovulation. These results can be used for prediction of ovulation and for FTAI in water buffalo. Our findings on follicular wave showed the dominant two wave pattern of follicular development and continued to grow up to its maximum size at ovulation time and suppress other subordinate follicles which is close to previous findings in cattle and swamp buffalo (Maylie and Hui, 1991). Ultrasound-guided follicle study of all follicles ≥4 mm in diameter during different stages of development resulted in the synchronous appearance of a new follicular wave within 1–2 days after ablation procedure in most of buffalo. Our findings indicated that follicle onset time was almost similar in two stages of experimental crossbred buffalo after ovulation (ovulation=day 0). The results were in accordance with the similar experiments performed on cattle (Garcia and Salaheddine, 1998).

Conclusion

Our findings on follicular wave revealed the significance of dominant two wave follicular development pattern that continued to grow up to its maximum size in crossbred buffalo We found that the onset of puberty was at 20~24 month age, this result similar in swamp type buffalo. The onset of puberty in crossbred buffalo heifers was strongly associated with dominant follicle diameter. Healthy postpartum crossbred buffalo first ovulation time was 27.7 ± 7.1 days, but uterine recovery time was 33.6 ± 2.4 days, we suggest that artificial insemination time in postpartum buffalo is at 48.5 ± 8.4 days which is the second ovulation time and is good higher pregnancy rate. These results highlighted the potential to improve the efficiency of estrus synchronization, super ovulation, fixed time artificial insemination, embryo transportation, increase pregnancy rate and distribution of genetic material in enhancing fertility rates in buffalo industry.

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