INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–169/2019/21–3–560–568
DOI: 10.17957/IJAB/15.0929
http://www.fspublishers.org

Full Length Article

Evaluation of Single and Multiple Follicular Dynamics in Chinese Crossbred Buffalo (Riverine × Swamp)

Adili Abulaiti[1], Hadeel Samy El-Qaliouby[1,3] and Liguo Yang[1,2*]

[1]*Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Education, 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 & Products, Wuhan, 430070, People's Republic of China*

[3]*Department of Animal Wealth Development, Animal Breeding and Production, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh, 13736, Qalyubia, Egypt*

[*]For correspondence: yangliguo2006@qq.com; adiliabulaiti@webmail.hzau.edu.cn; Hadil.Abbas@mail.hzau.edu.cn

Abstract

The aim of the study was to evaluate the single and multiple follicle development dynamics in three different physiological herds (pubertal, sexually matured and post-partum stages) of Chinese crossbred buffaloes (Nili-Ravi × Murrah × China indigenous swamp) and their relationship with Growth, regression, ovulation, initial and maximum size of dominant follicle (DF). 66 animals were ultrasonically examined throughout the estrous cycle. Firstly follicles were detected to be ≥4 mm in single and multiple follicle development at puberty, sexual maturity and post-partum stages. The results revealed that most of animals showed two waves of follicular dynamics during estrus cycle. These animals (n=59) were exposed to further study of follicular dynamics excluding animals showed one and three waves cycles. Postpartum buffaloes presented multiple follicle development has the significantly biggest length of estrus cycle (21.9±2.1) in comparison to sexually matured and Pubertal herds. However, there is detected no significant difference of estrus cycle length between single (20.1±2.8) and multiple (20.3±3.3) follicle development. Moreover, the number of follicle wave cycle is closely related to the estrus cycle length. The overall linear DF growth rate was significantly bigger ($P < 0.05$) in second wave multiple recruited follicles at post-partum period as compared to that of sexually matured and pubertal period. The biggest Initial and Maximum size found multiple recruited follicles at post-partum stage was 5.9±0.7 mm and 12.2±2.1 mm, respectively. Therefore, selecting the two wave cycle, with multiple recruited follicles will improve buffalo fertility by using MOET, superovulation and FTAI in Crossbred Buffaloes.

Keywords: Ultrasonography; Follicular dynamics; Crossbred buffalo; Puberty; Post-partum; Estrus cycle

Introduction

The understanding of follicular dynamics in buffalo is necessary for developing new techniques and improving the currently used regimens for the manipulation of the estrous cycle. Ovarian follicular dynamics in buffalo are similar to those of cattle. In order to improve reproductive efficiency of buffaloes, it is important to understand the process of follicle development, growth pattern, estrus and ovulation in Chinese crossbred buffalo. In cattle, follicular dynamics has been intensively studied in all reproductive status. During estrous cycle (Noseir, 2003), pregnancy (Ginther *et al.*, 1989), before puberty (Evans, 2003) and postpartum (Stagg *et al.*, 1995). But there are a fewer reports about follicular development in buffaloes. Buffalo industry in China has a huge potential for development and the Ministry of Agriculture is also intended to enhance the development of the buffalo to strengthen dairy production system which is weak in the southern industrial zone. Crossbreeding in Buffalo significantly increased the milk yield per animal but resulted in low fertility that has great impact to squeeze the development of the buffalo industry.

In China, there are mostly swamp buffaloes with low milk yield as compared to river buffaloes (Lu *et al.*, 2015). Crossbreeding of swamp with river buffaloes has proven to be an efficient strategy for improving milk yield (Cruz, 2014). Although rivers and swamp buffalo follicle development pattern research has been reported, but these two types of buffalo chromosome number is different, swamp buffalo 2n=48 with Murrah and Nili - Rafi buffalo river type 2n=50, produces polymorphic hybrid progeny (2n = 50, 2n = 49 and 2n = 48), containing 2n=49 chromosomes. Therefore, resulting in aneuploidy, lower reproductive performance, mainly silent estrus; heat

To cite this paper: Abulaiti, A., H.S. El-Qaliouby and L. Yang, 2019. Evaluation of single and multiple follicular dynamics in Chinese crossbred buffalo (Riverine × Swamp). *Intl. J. Agric. Biol.*, 21: 560–568

symptom is difficult to detect obviously, buffalo sexual maturity relatively late, the long time heat, irregular estrus ovulation; ovulation time is hard to predict, mating conception rate low, causing serious impact on the development of China's buffalo industry, in order to develop estrus ovulation, super ovulation technology, it is necessary to conduct the thorough research to the base of follicular growth rules (Das and Khan, 2010; Kumar *et al.*, 2011).

Follicular development in single ovulatory species proceeds through stages of recruitment, selection and dominance (Goodman and Hodgen, 1983). During the estrous cycle of buffaloes, follicular growth is characterized by development of one or two dominant an ovulatory follicles before maturation of a terminal ovulatory follicle. Each wave was characterized by the development of one large (dominant) follicle and a variable number of smaller (non-dominant) follicles (Ghuman *et al.,* 2016). Development of the first dominant follicle is the most consistent event related to follicular dynamics during the estrous cycle in buffaloes and cows (Savio *et al.*, 1988). In previous reports (Matton *et al.*, 1981; Staigmiller and England, 1982), it was suggested that a single large follicle inhibited the development of smaller follicles. Ovarian follicle growth in cattle culminates in the selection of a single dominant follicle which attains the ability for final maturation and ovulation once or twice during the luteal phase and at the end of the oestrous cycle (Mihm *et al.*, 2010). During the bovine estrous cycle, follicular development occurs in 2 or 3 waves (Matton *et al.*, 1981; Pierson and Ginther, 1988) and during each wave a single follicle becomes dominant whereas other follicles in the same wave regress (Savio *et al.*, 1988). The DF is different from other follicles because it can escape atresia (the fate of all other follicles), recruitment and selection, the dominance, processes that give rise to the DF (Lucy, 2007).

Improvement of Reproductive performance in dairy animals is a key to sustainable milk production. Ovarian follicle is a pivotal to reproduction and has a key role to regulate the estrous cycle (Channing, 1980; Campanile *et al.*, 2010). Therefore, understanding the follicular dynamics development and ovulation are needed to improve and control reproductive function in farm animals (Ireland *et al.*, 2000; Ali *et al.*, 2003; Das *et al.*, 2013). A good understanding of follicular dynamics can help us to alleviate various reproductive problems and to optimize the reproductive efficiency of buffalo. In the normal course of development, ovarian follicles progress through growing, static, and regression phases (Ginther *et al.*, 1989; Ireland *et al.*, 2000; Ali *et al.*, 2003; Honparkhe *et al.*, 2014; Mirmahmoudi *et al.*, 2014). Follicular dynamics are key areas of basic reproduction research (Yindee *et al.*, 2011). Buffalo has 1 to 3 follicular waves in each estrous cycle (Mondal *et al.*, 2008; Campanile *et al.*, 2010; Ginther *et al.*, 2015). However, 2-wave follicular dynamics is the predominant pattern in buffalo (Taneja *et al.*, 1995; Baruselli *et al.*, 1997; Manik *et al.*, 2002; Ali *et al.*, 2003; Presicce *et al.*, 2003; Warriach and Ahmad, 2007).

Follicular dynamics has received a considerable attention since last two decades, due to the importance of the animal reproduction. Still, many areas are needed to be explored. For successful buffalo breeding by using reproductive technology, further detailed study of follicular dynamics is needed to improve standard processes such as Multiple Ovulations, Embryo transfer and Fixed time artificial insemination, specifically suited to the buffalo which are still lacking (Honparkhe *et al.*, 2014). Although, there have been advancements in certain research areas but many still remained to be addressed. The current study was planned to evaluate the effect of single and multiple growing follicles on initial and maximum size of DF in both one wave and two wave estrous cycle and relationship with estrus cycle of crossbred buffaloes at pubertal, sexually mature and post-partum stage. Therefore, it is an urgent need to generate information about follicular dynamics in Chinese crossbreed buffaloes. In our study also identifies gaps in knowledge of reproductive physiology that could be considered important for the development of new technologies for assisted reproduction in buffaloes. understanding the exact mechanism of ovarian dynamics might open up new insight for the treatment of infertility which could improve the reproductive performance in buffalo.

Materials and Methods

Experimental Site and Animal Care

In our study the experimental animals were used with same breeding management, production, varieties, geographical area and conditions in hybrid crossbred buffalo that influence the follicular development and ovulation, but the above managemental conditions were differ from that reported before in previous literature.

Experimental Animals and their Care

This study was conducted at a private farm, Hubei Buffalo breeding base of Animal Husbandry Co., Ltd. in Jingmen, Shayang City, Hubei Province. The climate is subtropical monsoon with annual average temperature 16.1ºC and rainfall 949.4 mm. Initially 66 crossbred Chinese buffaloes (Murrah X NiliRavi X Chinese) were selected for experiment but later on 59 animals having two wave cycle were used to study the follicular dynamics. They were divided into three groups, namely pubertal herd (first ovulation animals; at the age of 18 to 23 months old, n=28 with mean body weight, chest girth, body height, body lengths and abdominal girths were 308.2±38.6 kg, 166.8±7.8 cm, 243.4±15.2 cm, 211.0±13.2 cm, 188.9±17.4 cm, respectively), sexual maturity herd (mature animals; at the age of 2.5 to 4 years old, n=21 with mean body weight, chest girth, body height, body lengths and abdominal girths were 433.0±72.1 kg, 186.9±12.9 cm, 136.1±5.8 cm, 128.9±5.8 cm, 120.6±5.5 cm respectively) post-partum stage (post-partum animals; at the age of 5 to 8 years old, n=17 with mean body weight, chest girth, body height, body lengths and abdominal girth were 588.1±89.5 kg, 212.0±11.7 cm, 132.3±4.9 cm, 128.1±5.1 cm and 120.3±4.7 cm, respectively). Animals were at adlib feed with total mixed ration (TMR) machine immersion silage corn and dry straw, supplemented by leguminous and non-leguminous green forage and concentrates.

Ultrasound Examination

Ultrasonography was performed as previously described (Gimenes *et al.*, 2011). Ovarian follicular dynamics were monitored twice a day at 9:00 a.m. and 3:00 p.m. with ultrasound equipment, (Desktop B-type Veterinary Ultrasound Scanner, WED - 9618 - V, equipped with LV2-3/6.5 MHz rectal probe). Back racking was done before inserting the probe. The transducer was placed over left and right ovaries alternatively and scanning was completed in many times to notice that all the follicles bigger than 4 mm in diameter. For orientation, the transducer was moved along the dorsal surface of the reproductive tract and then moved laterally to scan the ovaries. Each ovary was scanned several times. The picture was freeze and taken. The size of the multiple follicles were also observed and recorded. Diagrams of the relative positions of the follicle as well as related ovarian structures were drawn. To decrease mistake, all detections were completed by the same operator. Likewise, estrus cycle length, the number of follicular wave in a cycle and time of each wave and duration were documented.

A DF and its cohort were determined as a follicle wave (Knopf *et al.*, 1989). The day of follicle wave onset was observed as the day when the DF was first discover more than 4 mm in diameter in this study. The growth (in positive value) or atresia (in negative value) rates of dominant and subordinate follicles were calculated in a slope of linear relation among detection for follicle diameters. Follicle wave duration was calculated by interval in days between development onset and atresia of a DF.

This procedure was repeated for all follicles ≥5 mm in both ovaries. Follicle ablation was determined as collapse of the antral follicle following evacuation of follicular contents. A follicular wave was determined as the synchronous growth of groups of follicles (≥4 mm in diameter), which was followed by selection and continuous growth of the DF. The day of follicular wave emergence following ablation was retrospectively observed as the day when the DF was first observed at 4–5 mm in diameter. The day of selection of DF was determined as the day when difference in diameters of the dominant and subordinate follicles was first observed to be >4 mm. The growth rates of dominant and subordinate follicles were calculated by subtracting the diameter of the follicle at first observed from the maximum diameter of follicle divided by the number of days from observed to maximum diameter Ghuman *et al.*, 2016). Ovulation was discovered by the appearance of a DF on the next consecutive daily ultrasound detection. Follicle wave duration was calculated by interval in days between development onset and atresia of a DF.

Statistical Analysis

All the data is presented as mean±SEM of repeated experiments (n=3). Statistical analyses were accomplished using one way analysis of variance (ANOVA) to analyze the data by applying the statistical software (SPSS, 1997). Level of significance ($P < 0.05$) was observed by Duncan's multiple range tests. To analyze the effect of different factors on initial, increased and maximal diameter of DF, linear ordinary regression models were used. Significance was considered at ($P < 0.05$) (Warriach and Ahmad, 2007; Barkawi *et al.*, 2009; Honparkhe *et al.*, 2014). To examine the effect of different factors on emergence, increased and maximal diameter of DF, linear ordinary regression models according to (Chun-Qiang *et al.*, 2008; Valipour *et al.*, 2017) were used, namely $Y=X\alpha+X\beta+X\gamma+\mu j$, where Y indicates influencing factors, $X\alpha$ indicates ovary, $X\beta$ indicates physiological herd and $X\gamma$ denotes follicle wave, and μj value is a constant (Dimauro *et al.*, 2010; Hossein-Zadeh, 2016).

Results

Initial Diameter

For initial size of follicle emerging from single and multiple recruited follicles, results showed non-significant differences ($P < 0.05$) among puberty, sexually mature and post-partum groups having two-wave cycle. For initial follicular size, results revealed significant differences ($P < 0.05$) in post-partum stages for two-wave cycle among single and multiple growing follicles (Fig. 1). The greatest mean first follicle diameter emerging from single recruited follicle during first wave of 2-wave cycle was recorded (5.3±0.7 mm) in sexually mature groups, while emerging from multiple recruited follicles, the greatest mean initial follicular diameter was 5.5±0.7 mm in post-partum group. Likewise, the greatest average preliminary follicle size emerging from single and multiple recruited follicles during second wave of 2-wave cycle was recorded (5.6±0.5 mm) and (5.9±0.7mm) respectively in post-partum group (Fig. 1).

Maximum Diameter

Results confirmed that non-significant differences ($P < 0.05$) with respect to maximum size of DF emerging from single recruited follicle during first follicular wave among all three groups (Fig. 2). Maximum size (9.8±3.0 mm) of DF emerging from single recruited follicle during first wave was found in post-partum stage. However, emerging from multiple recruited follicle in second wave, outcomes showed

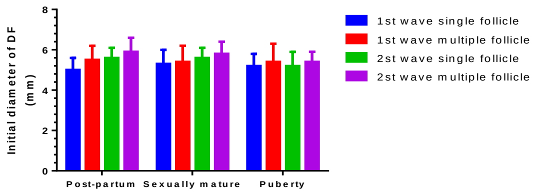

Fig. 1: Initial diameter of a dominant follicle on an ovary with single or multiple follicles recruited during the first and the second waves of a cycle of buffaloes at post-partum, sexually mature or puberty stage

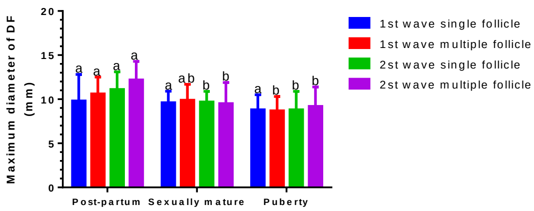

Fig. 2: Maximum diameter (mean ± SD, mm) of DF on an ovary with single or multiple follicles recruited during the first and the second waves of a cycle of buffaloes at post-partum, sexually mature or puberty stage

(Fig. 2) significant difference in post-partum group than sexually mature and puberty ($P < 0.05$). Maximum size of DF found in post-partum group was 11.1±2.0 mm. Likewise, emerging from multiple recruited follicles during first wave results showed the noteworthy difference in post-partum and pubertal group ($P < 0.05$). In second wave, significant difference was found in post-partum group than sexually mature and pubertal group ($P < 0.05$). The highest follicle diameter emerging from single and multiple recruited follicles during first and second wave, were observed in post-partum group (10.6±1.9 and 12.2±2.1 mm), respectively (Fig. 2).

Total Increase in Dominant Follicle (DF) Diameter from Onset to Ovulation

Total increased size of DF form onset to ovulation time for two wave cycle buffaloes has shown (Fig. 3). Results indicated that increased size difference of DF emerging from single follicle in puberty, sexually mature and post-partum groups in two-wave cycle was non-significant ($P <$ 0.05). Maximum size of DF during first wave was found in post-partum stage (5.2 ± 2.4 mm). However, in second wave DF was observed in sexually mature stage (5.4 ± 0.8 mm). Subsequently, increased size of DF, in single recruited follicle during first wave showed the significant difference in pubertal group than post-partum and sexually mature group ($P < 0.05$) but non-significant difference was perceived in DF size emerging from multiple recruited follicle. Highest DF increasing size emerging from single and multiple recruited follicles was found in post-partum group (5.2 ± 2.1 mm and 5.7 ± 2.1 mm) respectively.

Table 1: Length of Estrus cycle of buffaloes with single and multiple dominant follicles in a cycle at stage of postpartum, sexual mature or puberty

Physiological stage	Single follicle	Multiple follicle	Total
Post-partum (n=14)	21.4±2.4^{a}(n=5)	21.9±2.1^{a}(n=9)	21.7±2.2^{a}(n=14)
Sexually mature (n=20)	21.4±1.5^{a}(n=8)	21.6±2.0^{a}(n=12)	21.5±1.8^{a}(n=20)
Puberty (n=25)	19.1±3.0^{b}(n=18)	17.7±3.5^{b}(n=7)	18.6±3.2^{b}(n=25)
Total	20.1±2.8^{a}(n=31)	20.3±3.3^{a}(n=28)	20.2±3.0^{a}(n=59)

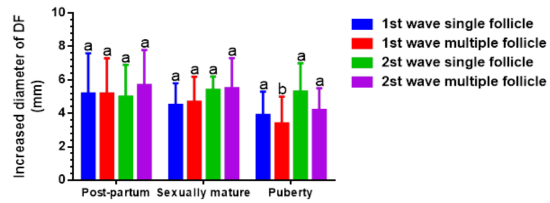

Fig. 3: Increase diameter of a DF on an ovary with single or multiple follicles recruited during the first and the second waves of a cycle of buffaloes at post-partum, sexually mature or puberty stage

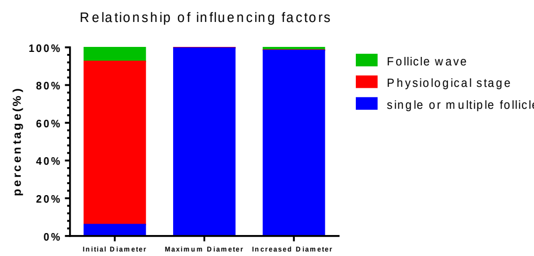

Fig. 4: Relationship of influencing factors to initial, maximum and total increase diameter of single and multiple DF

However, lowest DF size (3.4 ± 1.6 mm) emerging from multiple follicle recruitment was during first wave (Fig. 3).

Relationship of Influencing Factors to Initial, Maximum and Total Increase Diameter of DF

Among the three influencing factors (single and multiple follicle, physiological stage, follicle wave) single or multiple follicle 0.0142* (5.73) have highly significant effect (<0.05) on initial diameter of DF. follicular wave 0.000271*** (0.29) and Physiological stage0.000416*** (0.44) have highly significant effect (<0.05) on maximum diameter of DF, while single and multiple follicle 0.093316. (99.27). follicular wave0.012* (1.97) and Physiological stage3.94e-05*** (0.29) have highly significant effect (<0.05) on increasing diameter of DF had no major effect (Fig. 4).

Estrous Cycle Length with Respect to Single and Multiple Recruited Follicles

Estrous cycle length at pubertal stage with respect to single and multiple recruited follicles was significantly different ($P < 0.05$) from sexually mature and post-partum stages. Maximum, estrous cycle duration (21.9 ± 2.1days) in multiple recruited follicles was observed at post-partum

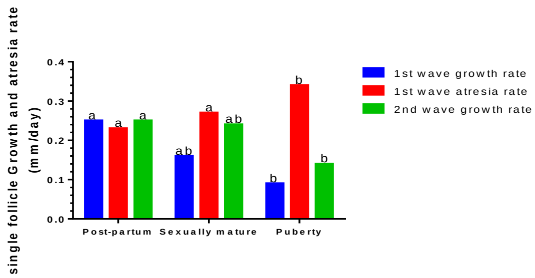

Fig. 5a: single follicle growth and atresia rate

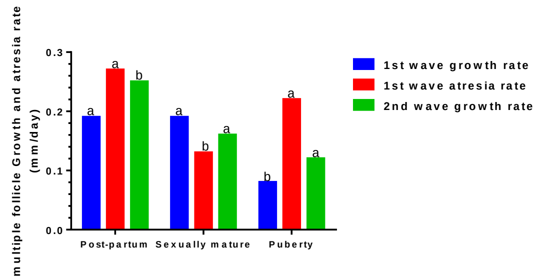

Fig. 5b: multiple follicle growth and atresia rate

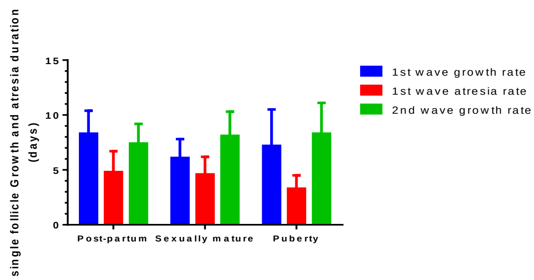

Fig. 5c: Single follicle growth and atresia duration

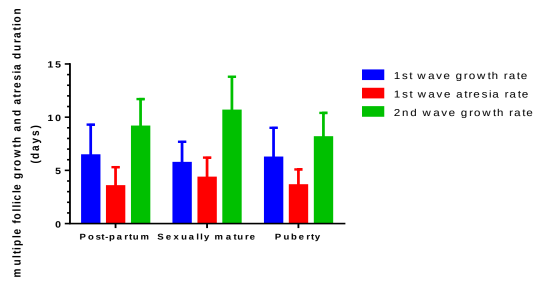

Fig. 5d: Multiple follicle growth and atresia duration

Fig. 5: The growth and atresia rate of DF in Post-partum, sexually mature and pubertal stages in single and multiple folliculogenesis in two follicle waves.

stage and was the lowest at puberty (17.7 ± 3.5 days) (Table 1).

The Growth and Atresia Rate of DF

DF growth and atresia results have been shown DF growth and atresia rate in 2-wave cycle for first wave in single follicle growing, results confirmed the significant differences ($P > 0.05$) in postpartum stage with puberty stage (Fig. 5a). DF Atresia rate in single growing follicle was significant ($P < 0.05$) in first wave for puberty stage than other two stages (Fig. 5a). Also significant difference ($P<0.05$) was observed in DF growth rate for single growing follicles in second wave post-partum and puberty stage (Fig. 5a). Greatest follicle growth duration days (8.3 ± 2.8 days) for DF of single follicle ovary was in puberty stage (Fig. 5c).

Likewise, DF growth rate in multiple growing follicles at puberty had shown considerable difference ($P < 0.05$) only in second wave than other two stages (Fig. 5b). Atresia rate of DF in multiple growing follicle had significant difference ($P < 0.05$) in first wave for puberty stage than other two stages (Fig. 5b). Whereas, longest follicle growth duration period for DF of multiple follicles was in second wave of sexually mature stage (10.6 ± 3.2 days) (Fig. 5d). DF growth rate in sexually mature stage for growing follicle was significantly different ($P < 0.05$) from single and multiple follicle growth rate (mm/d) during an estrous cycle (Fig. 5a, c and d). Single and multiple follicle growth and atresia duration showed no significant difference between all stages of buffalo ($P>0.05$) (Fig. 5c and d).

Relationship of Influencing Factors to Growth and Atresia Rate in Single or Multiple Developing Follicles and Estrous Cycle

Among all three factors in Fig. 6, results of follicular growth rate disclosed that physiological stage and follicular wave factors had highly significant (<0.05) effect. Follicle waves (0.85e-11***) (1.100000e-007) and physiological stage (1.63e-07***) (0.0002) displayed highest effect on growth rate for follicle. Likewise, effect on total Follicular growth, estrus cycle duration, results presented only follicular wave (2.8e-05***) (0.003) had noteworthy effect ($P < 0.05$), while physiological stage and single and multiple follicle had no major effect. Interestingly, results about the effect on follicular atresia, physiological stage (0.000536***) (0.1) and single and multiple follicle (8.85e-11***) (25.7) disclosed highly significant effect (<0.05) while ovary had insignificant effect. On the other hand, results about the effect on follicular atresia duration only follicular wave (2.8e-05***) (0.0000047) had noteworthy effect ($P < 0.05$), while physiological stage and single and multiple follicle had no major effect. Moreover, all three factors did not contribute to significant effect on estrus cycle length in follicular atresia (Fig. 6).

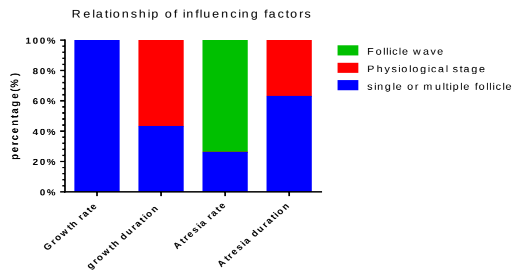

Fig. 6: Relationship of influencing factors to growth and atresia rate in single or multiple growing Follicles and Estrous Cycle

Discussion

This is the first complete description of ovarian follicular dynamics based on ultrasonographic analyses of development and regression of individual single and multiple follicle developing in Chinese crossbred buffaloes during the buffalo estrous cycle, it is characterized by 1 or 2 waves however, during each wave a single follicle becomes dominant whereas other follicles in the same wave regress. In our study we observed that most of animals were detected in two waves during an estrus cycle. The outcomes of the current study are similar to that reported by some authors of >80% of animals showed 2-wave pattern (Rajamahendran and Taylor, 1991; Bleach *et al.*, 2004; Warriach and Ahmad, 2007; Ginther *et al.*, 2015), on the other hand some author's reported a dominance >80% of the 3-wave pattern (Sirois and Fortune, 1988; Noseir, 2003). The difference might be because of hybrid vigor and different geographical location.

In our study, we observed that the highest DF increasing size emerging from single and multiple recruited follicles was found to be (5.2 ± 2.1 mm and 5.7 ± 2.1 mm) respectively, in post-partum group. The DF of a one-wave cycle demonstrated three distinct phases; growth phase, regression phase and re-growth phase resulting in ovulation. This pattern of follicular growth differs from the evolution of the ovulatory DF of two and three wave cycles which is defined by continuous growth without any regression until ovulation at the end of cycle as has been tested in buffaloes (Baruselli *et al.*, 1997; Manik *et al.*, 2002). The DF of a single wave cycle regressed considerably around mid-cycle after initial growth, while still holding its functional dominance. It is further confirmed that the same follicle become ovulatory after slow but steady growth from 15th day onwards. Alike growth configuration of the DF in one wave cycle has been stated by (Taneja *et al.*, 1995). The detection indirectly supplied the hypothesis that the size of the largest follicle alone is not a criteria to assess follicular dominance (Guilbault *et al.*, 1993; Baruselli *et al.*, 1997). In the current study three buffaloes demonstrated one wave follicular development with short estrous cycle length (9–15 days) conjectured that one wave cycle in buffaloes is linked with a short luteal phase and is defined by short estrous cycle length. Our findings showed that single and multiple follicle onset time was almost similar in all three stages of Chinese crossbred buffaloes after ovulation (ovulation=day 0) in the first wave. Similar findings have been reported in previous studies (Awasthi *et al.*, 2006; Satheshkumar *et al.*, 2015).

Present study revealed non-significant differences ($P >$ 0.05) between initial follicular size during two-wave cycle for both single and multiple growing follicles among post-partum, sexually matured and pubertal stages (Fig. 1). We examined Greatest mean initial follicle size emerging from single and multiple recruited follicles in second wave, it was noted as (5.6 ± 0.5 mm) and (5.9 ± 0.7 mm), respectively, in post-partum group. It has been well mentioned that the first follicular wave started around the day of ovulation in cattle which is defined by the presence of many small follicles at the same time (Madan *et al.*, 1994; Taneja *et al.*, 1995; Ginther *et al.*, 2015; Satheshkumar *et al.*, 2015). In buffaloes, a parallel pattern for the first follicular wave has been described which appears approximately on the day of ovulation as has been detected by ultrasonography and also by morphological studies (Madan *et al.*, 1994; Manik *et al.*, 1994; Baruselli *et al.*, 1997; Ali *et al.*, 2003; Azawi *et al.*, 2009). In the present work, maximum size of ovulatory follicles (OF) ranged between 9.8 ± 3.0 – 12.2 ± 2.1 mm in post-partum cases while in sexually matured and puberty stage, it was 9.7 ± 1.2 mm and 9.2 ± 2.2, respectively. which are close to lower range (10–18) mm that was recorded by (Khan and Das, 2012). Prior to the ovulation, diameter of the OF has been tested to be 12.7 ± 0.54 mm in post-partum Iranian river buffalo (Rastegarnia *et al.*, 2004).

The duration of the estrous cycle in buffalo is similar to that in cattle, ranging from 17 to 26 days with a mean of around 21 days (Jainudeen and Hafez, 2016). In our study we reported no significant difference of estrus cycle length between single (20.1 ± 2.8) and multiple (20.3 ± 3.3) follicle development. Similarly, two and three wave cycles length also differed with respect to the mean length of intervals between ovulation to be 22.27 ± 0.8 and 24.50 ± 1.88 d, respectively (Baruselli *et al.*, 1997). Buffalo having 3 wave cycle represent significantly longer inter ovulatory interval and greater length of estrous cycle (21.9 ± 2.1 days) as compared to those with 2 wave progression (17.7 ± 3.5 days). Comparable differences have also been described previously in cattle (Taylor and Rajamahendran, 1991) and in Murrah buffaloes (Baruselli *et al.*, 1997) between two and three wave succession. Generally, it is concluded that the number of follicular waves in each cycle is related with cycle length (Azawi *et al.*, 2009; Satheshkumar *et al.*, 2015), which is depending on the life span of the corpus luteum.

In our study, we observed that the single growing follicle growth rate showed significant difference ($P < 0.05$) in second wave cycles in post-partum and puberty stage (Fig. 5a), single and multiple follicle growth and atresia rate is higher in post-partum and sexually mature herds of buffaloes as compared to pubertal herd. The faster rate of growth of the DF among pubertal, sexually matured and post-partum animals seemed to be a consequence of the suppressive effect of progesterone on the estradiol negative feedback on the hypothalamus (Evans, 2003). DF growth rate and diameter is directly related with Gonadotropins (Rahe *et al.*, 1980). However, DF grows at a faster rate (Miura *et al.*, 2014) and its diameter is significantly greater at peak follicle time before ovulation (Baruselli *et al.*, 1997). In dairy buffalo, follicles reach at an ovulatory stage with a diameter of about 10 mm (Sartori *et al.*, 2004), only the dominant follicle (DF) of the last wave has regularly the chance for ovulation, whereas the other follicles undergo atresia. However, the growth rate of the ovulatory dominant follicle in the 2-wave cycles was greater ($P < 0.05$) than that in the single wave cycles (Taneja *et al.*, 1996).

This experiment elicits the clear relationship of the mean (±SD) maximum diameter of DF and growth and regression rate in different stages of buffaloes. We observed that DF growth rate in single growing follicle have significant difference ($P < 0.05$) in second wave cycle in post-partum and puberty stages. Daily growth rate ranged from 0.09 to 0.25 mm/day and 0.08 to 0.25 mm/day for single and multiple follicles, respectively similarly it is consistent with earlier studies which recorded daily growth rate of DF that ranged from 0.09 to 0.8 mm/day (Brito *et al.*, 2002; Awasthi *et al.*, 2006). Follicle wave dynamics is a well-controlled development that originates in a consistent and narrow period (Evans, 2003; Adams *et al.*, 2008; Carvalho *et al.*, 2016; Mokhtari *et al.*, 2016). However, DF grows abruptly at ovulation time for narrow period and its diameter was considerably greater at peak follicular time before ovulation (Awasthi *et al.*, 2006, 2007). Attaining a particular diameter is not the sole determinant of ovulation (Douville and Sirard, 2014), whereas acquisitions of both morphological and functional dominance are equally important in folliculogenesis (Khan and Das, 2012). Therefore, in dairy buffaloes, the assessing single and multiple follicle diameter in relation with growth and atresia rate can be a reliable indicator to precede fixed time AI (Khan and Das, 2012; Honparkhe *et al.*, 2014).

Conclusion

Chinese crossbreed buffaloes have predominantly two wave estrous cycle of 21.9±2 days, the assessing of DF diameter in relation to single and multiple recruited follicles in one and two wave cycles, DF follicle in two wave cycles appears to be a reliable indicator to precede for MOET, superovulation and fixed time AI. Prospective study should focus on simple adopt and impact oriented approaches, which determine the factors limiting fertility and estrus behavior in this commercially significant species. Despite the inherited problems in buffaloes, slow progress has been made in the application of assisted reproductive methods. AI is practiced commercially; in vitro embryo production, and embryo transfer remain in the realm of experimentation. If their prices are decreased these latest skills effort the opportunity to get more gain from the buffalo farming.

Acknowledgments

This work was supported by the National Key R&D Program of China (2017YFD0501903) and the earmarked fund for Modern Agro-industry Technology Research System (CARS-37-04B). National Natural Science Foundation of China (31772602, 31872352).

References

Adams, G.P., R. Jaiswal, J. Singh and P. Malhi, 2008. Progress in understanding ovarian follicular dynamics in cattle. *Theriogenology*, 69: 72–80

Ali, A., A.K. Abdel-Razek, S. Abdel-Ghaffar and P.S. Glatzel, 2003. Ovarian follicular dynamics in buffalo cows (*Bubalus bubalis*). *Reprod. Domest. Anim*., 38: 214–218

Awasthi, M.K., F.S. Kavani, G.M. Siddiquee, A.J. Dhami and N.P. Sarvaiya, 2007. Characterization of postpartum follicular development through ultrasound scanning and luteal function in suckled Mehsana buffaloes. *Ind. J. Anim. Sci.*, 77: 10–13

Awasthi, M.K., A. Khare, F.S. Kavani, G.M. Siddiquee, M.T. Panchal and R.R. Shah, 2006. Is one-wave follicular growth during the estrous cycle a usual phenomenon in water buffaloes (*Bubalus bubalis*). *Anim. Reprod. Sci*., 92: 241–253

Azawi, O.I., A.J. Ali and U.T. Noaman, 2009. A study on the ovarian follicular dynamic in Iraqi Northern Buffaloes. *Trop. Anim. Hlth. Prod*., 41: 79–83

Barkawi, A.H., Y.M. Hafez, S.A. Ibrahim, G. Ashour, A.K. El-Asheeri and N. Ghanem, 2009. Characteristics of ovarian follicular dynamics throughout the estrous cycle of Egyptian buffaloes. *Anim. Reprod. Sci*., 110: 326–334

Baruselli, P. S., R.G. Mucciolo, J.A. Visintin, W.G. Viana, R.P. Arruda, E.H. Madureira, C.A. Oliveira and J.R. Molero-Filho, 1997. Ovarian follicular dynamics during the estrous cycle in buffalo (*Bubalus bubalis*). *Theriogenology*, 47: 1531–1547

Bleach, E.C., R.G. Glencross and P.G. Knight, 2004. Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. *Reproduction*, 127: 621–629

Brito, L.F., R. Satrapa, E.P. Marson and J.P. Kastelic, 2002. Efficacy of PGF(2alpha) to synchronize estrus in water buffalo cows (*Bubalus bubalis*) is dependent upon plasma progesterone concentration, corpus luteum size and ovarian follicular status before treatment. *Anim. Reprod. Sci*., 73: 23–35

Campanile, G., P.S. Baruselli, G. Neglia, D. Vecchio, B. Gasparrini, L.U. Gimenes, L. Zicarelli and M.J. D'Occhio, 2010. Ovarian function in the buffalo and implications for embryo development and assisted reproduction. *Anim. Reprod. Sci.,* 121: 1–11

Carvalho, N.A.T.D., J G. Soares and P.S. Baruselli, 2016. Strategies to overcome seasonal anestrus in water buffalo. *Theriogenology*, 86: 200–206

Channing, C.P., 1980. Progesterone and estrogen secretion by cultured monkey ovarian cell types: influences of follicular size, serum luteinizing hormone levels, and follicular fluid estrogen levels. *Endocrinology*, 107: 342–352

Chun-Qiang, L.I., K.Q. Hong and L.I. Bao-Guo, 2008. Analysis of temporal-spatial variations of reference evapotranspiration during 1965 and 1999 in Hebei Province. *Chin. J. Agrometeorol*., 29: 414–419

Cruz, L.C., 2014. Transforming swamp buffaloes to producers of milk and meat through crossbreeding and backcrossing. *J. Anim. Plant Sci.*, 22: 157–168

Das, G.K. and F.A. Khan, 2010. Summer anoestrus in buffalo-a review. *Reprod. Domest. Anim*., 45: 483–494

Das, G.K., B.L. Kumawat and F.A. Khan, 2013. Ovarian follicular dynamics during estrous cycle and its aberrations during certain reproductive disorders in buffalo. *Theriogenol. Insight*, 3: 37–46

Dimauro, C., G. Catillo, N. Bacciu and N.P.P. Macciotta, 2010. Fit of different linear models to the lactation curve of Italian water buffalo. *Ital. J. Anim. Sci*., 4: 22–24

Douville, G. and M.A. Sirard, 2014. Changes in granulosa cells gene expression associated with growth, plateau and atretic phases in medium bovine follicles. *J Ovarian Res*., 7: 50

Evans, A.C.O., 2003. Characteristics of ovarian follicle development in domestic animals. *Reprod. Domest. Anim*., 38: 240–246

Ghuman, S.S., A.J. Hadiya, D.J.A.P.P.M. Lunagariya and K.K.A.C. Patel, 2016. Follicular dynamics during estrous cycle in postpubertal and postpartum gir cattle. *Ind. J. Anim. Reprod*., 37

Gimenes, L.U., N.A. Carvalho, M.F.S. Filho, F.S. Vannucci, J.R. Torres-Junior, H. Ayres, R.M. Ferreira, L.A. Trinca, E.S. Sartorelli, C.M. Barros, M.P. Beltran, G.P. Nogueira, R.J. Mapletoft and P.S. Baruselli, 2011. Ultrasonographic and endocrine aspects of follicle deviation, and acquisition of ovulatory capacity in buffalo (Bubalus bubalis) heifers. *Anim. Reprod Sci.* 123: 175–179

Ginther, O.J., J.P. Kastelic and L. Knopf, 1989. Intraovarian relationships among dominant and subordinate follicles and the corpus luteum in heifers. *Theriogenology*, 32: 787–795

Ginther, O.J., M.A. Siddiqui, J.M. Baldrighi, C.A. Wolf and J.M. Greene, 2015. Differences between follicular waves 1 and 2 in patterns of emergence of 2-mm follicles, associated FSH surges, and ovarian vascular perfusion in heifers. *Theriogenology*, 84: 853–861

Goodman, A.L. and G.D. Hodgen, 1983. The ovarian triad of the primate menstrual cycle. *In: Recent Progress in Hormone Research*, Vol. 39, pp: 1–73. Greep, R.O. (Ed.). Elsevier Inc

Guilbault, L.A., P. Rouillier, P. Matton, R.G. Glencross, A.J. Beard and PG. Knight, 1993. Relationships between the level of atresia and inhibin contents (alpha subunit and alpha-beta dimer) in morphologically dominant follicles during their growing and regressing phases of development in cattle. *Biol. Reprod*., 48: 268–276

Honparkhe, M., V.K. Gandotra, J.S. Matharoo, S.P. Ghuman, D. Dadarwal and J. Singh, 2014. Synchronization of follicular wave emergence following ultrasound-guided transvaginal follicle ablation or estradiol-17beta administration in water buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.*, 146: 5–14

Hossein-Zadeh, N.G., 2016. Modelling lactation curve for milk fat to protein ratio in Iranian buffaloes (*Bubalus bubalis*) using non-linear mixed models. *J. Dairy Res*., 83: 334–340

Ireland, J.J., M. Mihm, E. Austin, M.G. Diskin and J.F. Roche, 2000. Historical perspective of turnover of dominant follicles during the bovine estrous cycle: key concepts, studies, advancements, and terms. *J. Dairy Sci*., 83: 1648–1658

Jainudeen, M.R. and E.S.E. Hafez, 2016. Cattle and Buffalo. *In: Reproduction in Farm Animals*, 7th Edition, pp: 157–171. Hafez, B. and E.S.E. Hafez (eds.). John Wiley and Sons, Inc.

Khan, F.A. and G.K. Das, 2012. Follicular characteristics and intrafollicular concentrations of nitric oxide and ascorbic acid during ovarian acyclicity in water buffalo (*Bubalus bubalis*). *Trop. Anim. Hlth. Prod.*, 44: 125–131

Knopf, L., J.P. Kastelic, E. Schallenberger and O.J. Ginther, 1989. Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. *Domest. Anim. Endocrinol.*, 6: 111–119

Kumar, R., D. Kumar and B. Roy, 2011. Studies on repeat breeding of buffaloes. *Buff. Bull.,* 30: 177–183

Lu, Y., Y. Liao, M. Zhang, B. Yang, X. Liang, X. Yang, S. Lu, Z. Wu, H. Xu and Y. Liang, 2015. A field study on artificial insemination of swamp and crossbred buffaloes with sexed semen from river buffaloes. *Theriogenology*, 84: 862

Lucy, M.C., 2007. The bovine dominant ovarian follicle. *J. Anim. Sci*., 85: 350–374

Madan, M.L., M.S. Chauhan, S.K. Singla and R.S. Manik, 1994. Pregnancies established from water buffalo (*Bubalus bubalis*) blastocysts derived from *in vitro* matured, *in vitro* fertilized oocytes and co-cultured with cumulus and oviductal cells. *Theriogenology*, 42: 591–600

Manik, R.S., M.L. Madan and S.K. Singla, 1994. Ovarian follicular dynamics in water buffaloes (*Bubalus bubalis*): Ultrasonically monitoring individual follicles for wave hypothesis. *Theriogenology,* 41: 246

Manik, R.S., P. Palta, S.K. Singla and V. Sharma, 2002. Folliculogenesis in buffalo (*Bubalus bubalis*): A review. *Reprod. Fert. Dev*., 14: 315–325

Matton, P., V. Adelakoun, Y. Couture and J.J. Dufour, 1981. Growth and replacement of the bovine ovarian follicles during the estrous cycle. *J. Anim. Sci*., 52: 813

Mihm, M., M.A. Crowe, P.G. Knight and E.J. Austin, 2010. Follicle wave growth in cattle. *Reprod. Domest. Anim.*, 37: 191–200

Mirmahmoudi, R., M. Souri and B.S. Prakash, 2014. Endocrine changes, timing of ovulation, ovarian follicular growth and efficacy of a novel protocol (Estradoublesynch) for synchronization of ovulation and timed artificial insemination in Murrah buffaloes (*Bubalus bubalis*). *Theriogenology*, 81: 237–242

Miura, R., S. Haneda, H.H. Lee, A. Miyamoto, T. Shimizu, K. Miyahara, Y.I. Miyake and M. Matsui, 2014. Evidence that the dominant follicle of the first wave is more active than that of the second wave in terms of its growth rate, blood flow supply and steroidogenic capacity in cows. *Anim. Reprod. Sci*., 145: 114–122

Mokhtari, A., M. Kafi, M.J. Zamiri and R. Akbari, 2016. Factors affecting the size of ovulatory follicles and conception rate in high-yielding dairy cows. *Theriogenology*, 85: 747–753

Mondal, S., B.S. Prakash and P. Palta, 2008. Relationship between peripheral plasma inhibin and FSH concentrations in Sahiwal cows (*Bos indicus*) and Murrah buffaloes (*Bubalus bubalis*) during estrous cycle. *Trop. Anim. Hlth. Prod.*, 40: 403–406

Noseir, W.M., 2003. Ovarian follicular activity and hormonal profile during estrous cycle in cows: the development of 2 versus 3 waves. *Reprod. Biol. Endocrinol*., 1: 50

Pierson, R.A. and O.J. Ginther, 1988. Follicular populations during the estrous cycle in heifers III. Time of selection of ovulatory follicle. *Anim. Reprod. Sci.,* 16: 81–95

Presicce, G.A., A. Parmeggiani, E.M. Senatore, R. Stecco, V.L. Barile, G.J.D. Mauro, G.D. Santis and G.M. Terzano, 2003. Hormonal dynamics and follicular turnover in prepuberal Mediterranean Italian buffaloes (*Bubalus bubalis*). *Theriogenology*, 60: 485–493

Rahe, C.H., RE. Owens, J.L. Fleeger, H.J. Newton and P.G. Harms, 1980. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. *Endocrinology*, 107: 498–503

Rajamahendran, R. and C. Taylor, 1991. Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Holstein heifers treated with norgestomet. *J. Reprod. Fertil*., 92: 461–467

Rastegarnia, A., A. Niasari-Naslaji, P. Hovareshti, F. Sarhaddi and M. Safaei, 2004. The effect of different doses of gonadorelin on ovarian follicle dynamics in river buffalo (*Bubalus bubalis*). *Theriogenology*, 62: 1283–1291

Sartori, R., J.M. Haughian, R.D. Shaver, G.J.M. Rosa and M.C. Wiltbank, 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J. Dairy Sci*., 87: 905–920

Satheshkumar, S., K. Brindha, A. Roy, T.G. Devanathan, D. Kathiresan and K. Kumanan, 2015. Natural influence of season on follicular, luteal, and endocrinological turnover in Indian crossbred cows. *Theriogenology*, 84: 19–23

Savio, J.D., L. Keenan, M.P. Boland and J.F. Roche, 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. *J. Reprod. Fert*., 83: 663–671

Sirois, J. and J.E. Fortune, 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol. Reprod.*, 39: 308–317

SPSS, 1997. *Statistical Package for Social Sciences*. SPSS for Windows. Release 10.0 SPSS Inc., USA

Stagg, K., M.G. Diskin, J.M. Sreenan and J.F. Roche, 1995. Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. *Anim. Reprod. Sci.,* 38: 49–61

Staigmiller, R.B. and B.G. England, 1982. Folliculogenesis in the bovine. *Theriogenology,* 17: 43–52

Taneja, M., A. Ali and G. Singh, 1996. Ovarian follicular dynamics in water buffalo. *Theriogenology*, 46: 121–130

Taneja, M., G. Singh, SM. Totey and A. Ali, 1995. Follicular dynamics in water buffalo superovulated in presence or absence of a dominant follicle. *Theriogenology*, 44: 581–597

Taylor, C. and R. Rajamahendran, 1991. Follicular dynamics and corpus luteum growth and function in pregnant versus nonpregnant cows. *J. Dairy Sci*., 74: 115–123

Valipour, M., M.A.G. Sefidkouhi and M. Raeini−Sarjaz, 2017. Selecting the best model to estimate potential evapotranspiration with respect to climate change and magnitudes of extreme events. *Agric. Water Manage*., 180: 50–60

Warriach, H.M. and N. Ahmad, 2007. Follicular waves during the oestrous cycle in Nili-Ravi buffaloes undergoing spontaneous and PGF2alpha-induced luteolysis. *Anim. Reprod. Sci*., 101: 332–337

Yindee, M., M. Techakumphu, C. Lohachit, S. Sirivaidyapong, A. Na-Chiangmai, H. Rodriguez-Martinez, G.C.V.D. Weyden and B. Colenbrander, 2011. Follicular Dynamics and Oestrous Detection in Thai Postpartum Swamp Buffaloes (*Bubalus bubalis*). *Reprod. Domest. Anim*., 46: 91–96

(Received 13 August 2018; Accepted 25 September 2018)