



Full Length Article

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

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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. © 2019 Friends Science Publishers

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 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, buffalo sexual maturity relatively late, the long time heat, heat symptom is difficult to detect obviously, irregular estrus ovulation, estrus detection is difficult to master, 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 (Sirois and Fortune, 1988). 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.*, 2002). 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; Sirois and Fortune, 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 (Luey, 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). Results indicated the presence of either one or two waves of follicular growth per cycle. Each wave was characterized by the development of 1 large (dominant) follicle and a variable number of smaller (nondominant) follicles (Taneja *et al.*, 1996).

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 crossbred 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

Experimental purposes: 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 managerial conditions were differ from that reported before in previous literature.

Experimental Animals

This study was conducted at a private farm, Hubei Buffalo breeding base of Animal Husbandry Co., Ltd. in Jingmen, Shayang City, Hubei Province. Geographical location of experimental site was East longitude 111° 51 '112° 42' and North latitude 30° 23 'between 55'. The climate is subtropical monsoon with annual average temperature 16.1°C 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 (5-8 years, n=17), maturity stage (2.5 to 4 years old, n=28) and pubertal stage (at the age of 18 to 23 months old, n=21). Animals were at adlib feeding 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 daily at 9:00 am and 3:00 pm 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 (L *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 group 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 (Sirois and Fortune, 1988). Ovulation was discover 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 \pm 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 Inc., 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.*, 2013; Valipour, 2014, 2016; Valipour *et al.*, 2017) were used, namely $Y = X\alpha + X\beta + X\gamma + \mu_j$, where Y indicates influencing factors, X α indicates ovary, X β indicates physiological herd and X γ denotes follicle wave, and μ_j value is a constant (Dimauro *et al.*, 2010; Das *et al.*, 2012; 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 group 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 group, while emerging from multiple recruited follicles, the greatest mean initial follicular diameter recorded 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±0.7 mm) 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. 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 ($P < 0.05$).

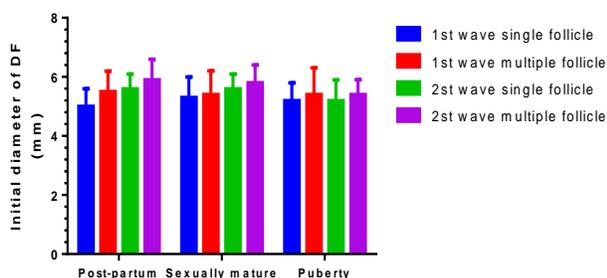


Fig. 1: Initial diameter of a dominant follicle on an ovary with single or multiple follicles recruited during the first and the second waves of a cycle of buffaloes at post-partum, sexually mature or puberty stage

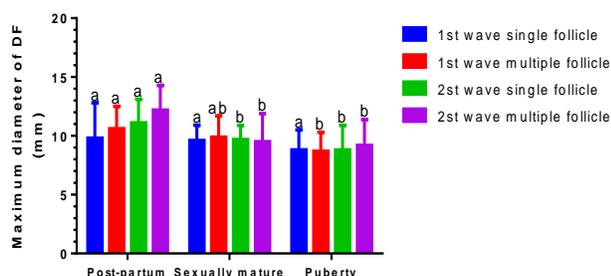


Fig. 2: Maximum diameter (mean \pm SD, mm) of DF on an ovary with single or multiple follicles recruited during the first and the second waves of a cycle of buffaloes at post-partum, sexually mature or puberty stage

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 mm 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 from 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. However,

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 \pm 2.4 ^a (n=5)	21.9 \pm 2.1 ^a (n=9)	21.7 \pm 2.2 ^a (n=14)
Sexually mature (n=20)	21.4 \pm 1.5 ^a (n=8)	21.6 \pm 2.0 ^a (n=12)	21.5 \pm 1.8 ^a (n=20)
Puberty (n=25)	19.1 \pm 3.0 ^b (n=18)	17.7 \pm 3.5 ^b (n=7)	18.6 \pm 3.2 ^b (n=25)
Total	20.1 \pm 2.8 ^a (n=31)	20.3 \pm 3.3 ^a (n=28)	20.2 \pm 3.0 ^a (n=59)

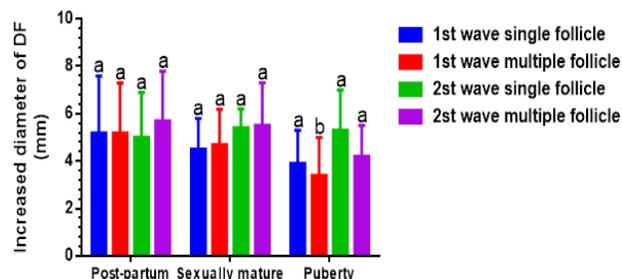


Fig. 3: Increase diameter of a DF on an ovary with single or multiple follicles recruited during the first and the second waves of a cycle of buffaloes at post-partum, sexually mature or puberty stage

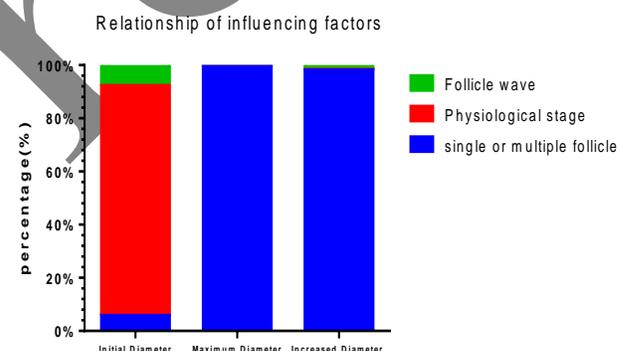


Fig. 4: Relationship of influencing factors to initial, maximum and total increase diameter of single and multiple DF

lowest DF size (3.4 ± 1.6 mm) emerging from multiple follicle recruitment was seen 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 stage 0.000416*** (0.44) have highly significant effect (< 0.05) on maximum diameter of DF, while single and multiple follicle 0.093316. (99.27). follicular wave 0.012* (1.97) and Physiological stage 3.94e-05*** (0.29) have highly significant effect (< 0.05) on increasing diameter of DF. had no major effect (Fig. 4).

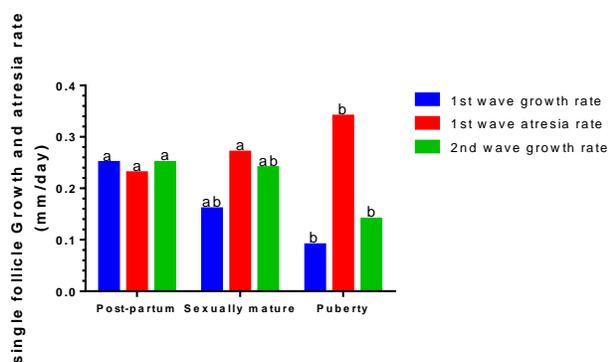


Fig. 6a: single follicle growth and atresia rate

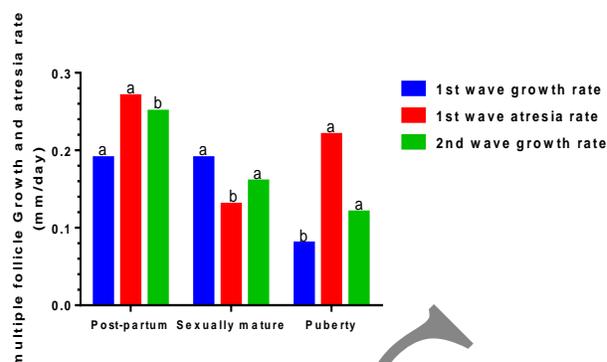


Fig. 6b: multiple follicle growth and atresia rate

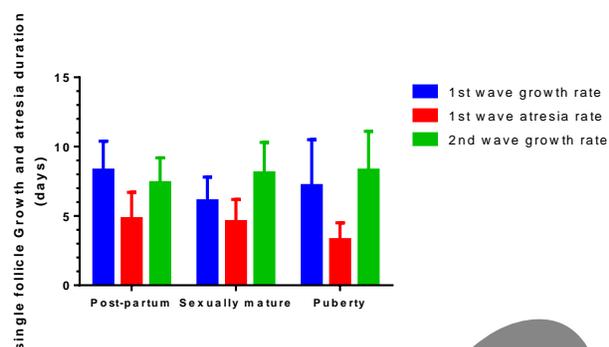


Fig. 6c: Single follicle growth and atresia duration

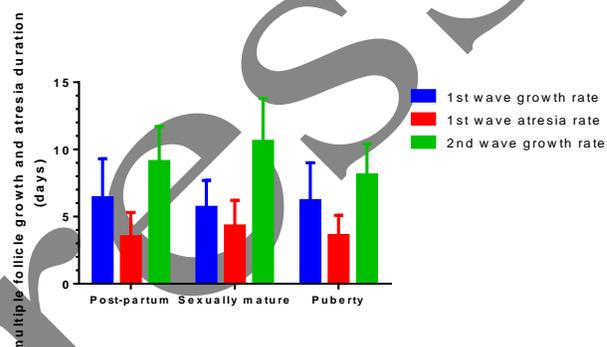


Fig. 6d: multiple follicle growth and atresia duration

Fig. 6: The growth and atresia rate of DF in Post-partum, sexually mature and pubertal stages in single and multiple folliculogenesis in two follicle waves

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 ± 2.1 days) in multiple recruited follicles was observed at post-partum stage and was the lowest at puberty (17.7 ± 3.5 days) (Fig. 5).

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. 6a). DF Atresia rate in single growing follicle was observed significance difference ($P < 0.05$) in first wave for puberty stage than other two stages (Fig. 6a). DF growth rate in single growing follicle was observed significance difference ($P < 0.05$) in second wave Post-partum and puberty stage (Fig. 6a). Greatest follicle growth duration days (8.3 ± 2.8 days) for DF of single follicle ovary was in puberty stage (Fig. 6c).

Likewise, DF growth rate in multiple growing follicle at puberty had seen considerable difference ($P < 0.05$) only in second wave than other two stages (Fig. 6b). 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. 6b). 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. 6d). 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. 6a, c and d). Single multiple follicle growth and atresia duration no significance difference all stage of buffalo ($P > 0.05$) (Fig. 6c 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. 7, 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,

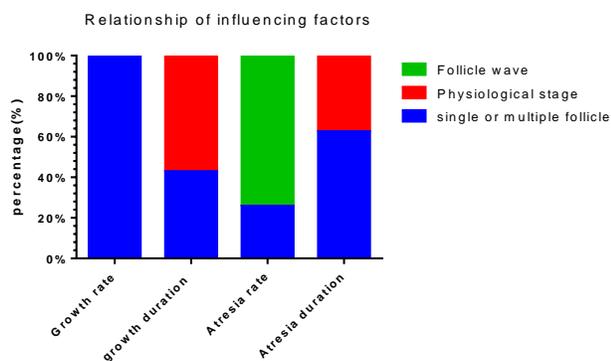


Fig. 7: Relationship of influencing factors to growth and atresia rate in single or multiple growing Follicles and Estrous Cycle

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 all three factors did not contribute to significant effect on estrus cycle length in follicular atresia (Fig. 7).

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 author's of about $> 80\%$ of animals showed 2-wave pattern (Ginther *et al.*, 1989; 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 described 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. Analogous 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 recorded 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.*, 1996). 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. 6a), 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 (\pm 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; de 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 crossbred 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; embryo transfer, 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.

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