Superovulatory Response in Relation to Follicular Dynamics and Presence of Dominant Follicles in Egyptian Buffaloes

Bassiouni Heleil and Yasser El Deeb

Depatment of Theriogenology, Faculty of Veterinary Medicine,
Kafrelsheikh, University, 33516 Kafr-Elshseikh, Egypt

Abstract: The present study was designed to investigate the pattern of follicular dynamics during the estrous cycle, for evaluation the superovulatory response of different follicular wave cycles as well as studying the effect of presence or absence of a dominant follicle on the superovulatory response in Egyptian buffaloes. A total of 30 buffalo was used in the present study. Three experiments were conducted: in experiment1, the growth, selection and ovulation of ovarian follicles were ultrasonically monitored in 30 animals after estrous synchronization by using PGF2α .Experiment 2 was conducted to examine superovulatory response in different follicular wave cycles ,while experiment 3 was performed to evaluate the effect of presence or absence of a dominant follicle at the time of gonadotrophin injection on the superovulatory response. Results indicated that the 30 examined buffalos have estrous cycle with one(6.6%), two(53.3%) or three 3(40%) follicular waves and that the number of waves in the cycle is associated with the luteal phase and with the estrous cycle length. Moreover, there were no significance differences in the superovulatory response between two and three wave cycles. However, the numbers of corpora lutea, recovered embryo and transferable embryo were higher in buffalo with non-dominant than with dominant follicles (4.64±0.2 vs 3.36±0.23, 2.35±0.22 vs 1.09±0.19 and 1.4±0.17 vs 0.63±0.15, respectively). In conclusion, the present results showed that the out come of superovulatory response in buffaloes can not be influenced by type of follicular dynamics, Moreover, the feature of ovarian status at initiation of gonadotrophin treatment tended to be related to superovulatory response in the presence or absence of a dominant follicle.

Key word: Buffalo · Superovulation · Follicular dynamics · Dominant follicle.

INTRODUCTION

Embryo transfer (ET) technique has been applied for enlarging progeny population of high genetic merit dams in animal breeding programs, especially for animals with low reproductive rate such as buffaloes [1]. Traditionally, ET relies on superovulation technique to maximize reproductive performance of the dam [2]. Superovulation is still widely used to produce valuable bovine embryo for breeding. Enormous efforts have been devoted to investigate the factors influencing the superovulatory response in buffaloes. However, the progress has been limited in view of ovulation rate and yield of viable embryos. Various factors were reported to influence the superovulatory response in buffaloes. These factors include factors extrinsic to the animals, such as sources and purities of gonadotrophin and their administration [3,4], and intrinsic factors related to the ovarian status, especially the stage of the ovarian follicular waves at the time of treatment [5].

Since the advent of ultrasound technology applied to animal reproduction, a large amount of information has been collected about ovarian follicular dynamics in domestic animals[6, 7]. Ovarian follicular dynamics have been studied by several authors in buffalo species[8-10]. Ovarian follicular growth in buffaloes was similar to that observed in cattle and was characterized by waves of follicular recruitment, growth and regression [9], The studies by Taneja *et al.* [8] confirmed the results of Danell [11] that development of ovarian follicles occur in one or two waves per the estrous cycle. Three wave cycles were reported in Murrah buffaloes [9] and the two wave cycles were the most common. During each follicular wave the dominant follicles developed and through the production of steroidal and non steroidal substances suppress the development of subordinate follicles and

---

Corresponding Author: Bassiouni Heleil, Depatment of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh,University, 33516 Kafr-Elshseikh, Egypt

prevents the emergence of the next follicular wave. Thus, the presence of a dominant follicle at initiation of gonadotrophin treatment has been known to decrease the superovulatory response [5].

The current study was designed to study (i) Characteristics of follicular dynamic during the estrous cycles, (ii) the superovulatory response of different follicular wave cycles and (iii) the effect of presence or absence of a dominant follicle on the superovulatory response in buffaloes.

MATERIAL AND METHODS

Animals: Thirty buffalo cows, 7-10 years of age, weighting 450 to 550 kg and having displayed at least two estrous cycles of normal duration were used in this study. The study was conducted in buffaloes herd selected from Mehalt Mousa farm belonging to Animal Production Research Institute at Kafr El sheikh province, Egypt during the period from October, 2009 to March, 2010. Animals were apparently healthy and with good body condition. They were fed on hay, silage, concentrate mixture, barseem and rice straw.

Ultrasonography: Transrectal ultrasonography was carried out using ultrasound scanner supplied with 5 MHZ array transducer (Ultra scan 90 alliance, Quebec, C anda). Animals were examined ultrasonography to monitor ovarian follicular dynamics, as well as to investigate and demonstrate the response to superovulatory response [12].

Experimental Protocols
Experiment 1: To determine the characteristics of the follicular dynamics during estrous cycles, a total of 30 buffaloes were monitored by ultrsonography after estrous synchronization by i.m injection of two doses each of 2 ml estrumate (500ug cloprostenol, Schering animal health, Co, Germany), at an interval of 11 days. The ovaries were scanned daily each morning, after estrous detection, for an entire estrous cycle from an ovulation to the next. All observations were carried out by the same person and were recorded on video-tape. The video- tape was subsequently reviewed on the screen of ultra sound scanner, and diagrams depicting the relative location of follicles were made for each ovary and their growth were monitored individually. Follicles were measured with a ruler calibrated against the in- built scale provided with the ultrasound unit [13]. This procedure allowed to follow the pattern of growth and selection of individual follicles during the entire estrous cycle while maintaining the identities of individual follicles [14].

Experiment 2: It was conducted to determine the superovulatory response of different follicular wave types. Buffalo having two (group 1, n=16) and three (group2, n=12) follicular waves from the first exp. were superovulated by i.m injection of Pregnant mare serum gonadotrophin (Folligon, Intervet Co, B.V.Hol and) with a dose of 3000 I U [15] on day 10 of natural estrous cycles. Luteolysis was induced by administration of 2 ml estrumate i.m at 48 h after initiating gonadotrophin treatment. Number of small follicles ($\leq$4mm) and large follicle($\leq$8mm) were determined by using ultrasonography before and during the superovulatory treatment . All animals were artificially inseminated two times with frozen semen at 12h. Intervals following detection of st anding heat. Embryo recovery was non-surgically performed on day 5-6 [16]. It was conducted by using sterile two way Foley catheter (size 8-22). The flushing media was phosphate buffer saline (PBS) containing 1%. Bovine serum albumin (BSA). Efficacy of superovulation was determined by estimating number of corpora lutea by ultrasonography.

Experiment 3: It was performed to investigate the effect of presence or absence of a dominant follicle at the start of gonadotrophin treatment on the superovulatory response. Ultrasonographic examination of the ovaries during experiment 2 indicated that buffaloes were assigned to two groups according to the presence (G1=11) or absence (G2=17) of a dominant follicle ($\geq$10 mm) [5] at the start of superovulation regardless the type of follicular wave. Twenty eight buffalo were superovulated as mentioned before in experiment 2.

STATISTICAL ANALYSES

The obtained data were represented as mean + SEM. T test was used for comparison of mean values of the various treatments. All data were statistically analyzed according to *Snedecor and Cochran* [17].

RESULTS

Experiment 1: The results indicated the presence of 3 patterns of ovarian follicular waves :one wave (n=2,6.6%), two waves (n=16,53.3%) and three waves (n=12,40%). As shown in Table 1 , buffaloes that presented three waves of follicular growth also presented the longest

Table 1: Charachteristics of the follicular dynamics during estrous cycles in Egyptian buffaloes.

Parameter	Number of waves(NW)		Significance
	Two wave	Three wave	
Number of buffaloes	16/30	12/30	
Length of estorus cycle(day)	20.69±0.28	23.00±0.33	***
Length of luteal phase(day)	10.13±0.31	12.58±0.26	***
Emergence of first wave(day)	1.14±0.06	0.84±0.04	***
Emergence of second wave(day)	11.00±0.29	8.62±0.29	***
Emergence of third wave(day)	--	16.31±0.31	
Number of recruited follicles in 1s wave ($\geq$4mm)	7.13±0.22	8.08±0.26	**
Number of recruited follicles in 2nd wave($\geq$4mm)	6.31±0.20	6.17±0.21	ns
Number of recruited follicles in 3rd wave($\geq$4mm)	--	5.42±0.26	
Persistence of 1st dominant follicles(day)	19.31±0.31	17.00±0.48	***
Persistence of 2nd dominant follicles(day)	10.81±0.31	13.31±0.33	***
Persistence of 3rd dominant follicles(day)	--	7.69±0.29	
Maximum diameter of 1st dominant follicles(mm)	14.69±0.34	12.57±0.33	***
Maximum diameter of 2n dominant follicles(mm)	15.06±0.27	12.83±0.32	***
Maximum diameter of 3rd dominant follicles(mm)	--	12.85±0.37	

Values are means±st andard errors.

Significance levels:**,p<0.01;***,p<0.001;ns,not significant p<0.05.

Table 2: Superovulatory response in buffaloes with two and three follicular wave cycles

Parameters	G1(n=16)	G1(n=12)	Significance
No.follicles$\geq$4mm(day0)	6.18± 026	6.08±0.29	ns
No.of follicles$\geq$8mm in the day post treatment	7.50± 0.30	7.33±0.38	ns
No. of ovulation	5.81± 0.36	6.08±0.31	ns
No.of corpora lutea	3.94± 0.31	4.42±0.31	ns
No. of unovulates follicles	1.76± 0.18	1.75±0.22	ns
No.of recovered embryo	1.93± 0.34	1.92±0.42	ns
No. of unfertilized ova	0.76± 0.20	0.77±0.23	ns
No. of transferable embryo	1.06± 0.21	1.17±0.30	ns

Values are means± st andarderrors Significance levels:**,p<0.01;***,p<0.001;ns, not significant p<0.05.

Table 3: Effect of dominant follicles at the time of superovulatory treatment on superovulatory response in buffaloes

parameters	G 1(n=11)	G2(n=17)	Significance
No. of small antral follicles=4mm	6.09±0.37	6.17±0.23	ns
No. of follicles=8mm	6.71±0.30	7.87±0.24	**
No. of ovulation	5.00±0.27	6.52±0.26	***
No .of corpora lutea	3.36±0.23	4.64±0.20	***
No .of recovered embryo	1.09±0.19	2.35±0.22	***
No. of transferable embryo	0.63±0.15	1.4±0.17	**

Values are means± standarderrors Significance levels:**,p<0.01;***,p<0.001;ns, not significant p<0.05.

luteal phase and estrous cycles. There were significant differences between two and three wave cycles with regard to the day of emergence of the first and second waves (p<0.001) and the number of recruited follicles ($\leq$4mm) in the first wave(p<0.01). However, there was no difference between two and three wave cycles in the number of recruited follicles ($\leq$4mm) in the second wave. Two and three wave cycles differed significantly (p<0.001) with regard to persistence of the first and second dominant follicles , and in the maximum diameters of both first and second dominant follicles

Experiment 2: Table 2 reveals the superovulatory response of different follicular wave cycles in buffalo. There were no significant differences between two(G1) and three(G2) wave cycles in the number of follicles $\geq$4mm at the start of superovulatory response and follicles $\geq$8mm in the day post superovulatory treatment. Non significant differences were also found between two and three wave cycles in the mean numbers of ovulations , corpora lutea, unovulated follicles, recoverd embryos, unfertilized ova and transferable embryo.

Experiment 3: Table 3 reveals the effect of dominant follicles at the time of superovulatory treatment on the superovulatory response. Number of small follicles (≥4mm) did not differ significantly between buffaloes of dominant(G1) and non dominant (G 2) follicles. A greater number (p<0.01) of large follicles (≥8mm) was recorded post superovulatory treatment in buffalo superovulated in the absence of dominant follicles. Mean numbers of ovulation ,corpora lutea, recovered embryo and transferable embryos were significantly higher (p<0.001) in buffaloes of non dominant than dominant group.

DISCUSSION

Superovulation is crucial for successful application of ET. These experiments studied the effect of follicular dynamics and dominant follicles on superovulatory response in buffaloes for achieving good superovulation results and improve embryo yield to maximum utilization of the reproductive potential of high merit buffaloes

The results of present study demonstrated that ovarian follicles developed in a wave like fashion in Egyptian buffaloes similar to those observed in Murrah buffaloes [8, 9, 18] and in cattle [14, 19]. In the present study, three patterns of follicular growth were observed during estrous cycles. One (n=2,6.6%), two (n=16,53.3%) and three (n=12,40.0%) follicular waves per cycles were recorded in almost equal proportion in accordance with the reports of Baruselli *et al.* [9] and Azawil *et al.* [20]. Non of the animals exhibited a four follicular wave cycle in the present study. In contrast, the majority of Murrah buffaloes showed one or two wave cycles [8], but Warrich and Ahmed [21] reported that the majority of buffalo in Pakistan had three and four waves pattern of follicular growth. The present findings explained that, the number of waves of follicular growth is associated to the duration of estrous cycle and luteal phase. Buffalo that presented three waves of follicular growth also presented the longest luteal phase and estrous cycle. These results agree with the result of Baruselli *et al.* [9] and Presicce *et al.* [10] in buffalo and Fortune *et al.* [19] and Knopf *et al.* [22] in cattle. Our results indicated that there were significant difference between maximum diameter of both the first dominant follicle (14.69±0.34 vs 12.57±0.33) and the second follicle(15.06±0.27vs 12.83±0.32) for two and three wave cycles, respectively. Terzano *et al.* [23] also reported that, the diameter of ovulatory follicles ranging between 13mm and 15mm in buffalo. In cattle, previous studies were also agree with these results [24,25].

The results of the present study showed that the first and second waves appeared earlier in the estrous cycle with three waves than with two waves. The same results was obtained in cattle by Fortune [24]. However, Baruselli *et al.* [9] explained that there were no difference between second and third wave cycles with regard to the day of emergence of the first wave in buffalo. Since the day of onset of the second wave ranged widely between buffaloes, it is difficult to set up a superovulation schedule. In fact, studies in cattle [26, 27] have demonstrated that there is a positive effect on the superovulatory response when initiated at the time of wave emergence, i.e. near the expected time of the endogenous wave eliciting FSH surge.

In comparison of superovulatory response of animals with two waves versus animals with three waves, our results revealed no differences in the number of ovulations or the number of recovered embryo in buffalo in which superovulatory treatment were initiated on the day of emergence of second wave(10 day of cycle). These confirmed by our results in experiment 1,whereas the number of recruited follicles in 2nd wave was similar for the buffaloes with two wave and three wave cycles. Our results were in accordance with Monniaux *et al.* [28] who reported that luteal response to PMSG stimulation depend on total number of growing follicles in the ovaries at the time of initiation of superovulation. Also Singh *et al.* [29]recorded similar results in cattle.

Concerning the result of experiment 3 of the current work, there was an increase in the mean numbers of corpora lutea, recovered and transferable embryos when superovulatory treatments were initiated in the absence of a dominant follicle. The present findings were in accordance with the findings of Taneja *et al.* [5] who found that number of corpora lutea at palpation per rectum was higher in superovulated buffalo of the non dominant than the dominant group. However, the same authors explained that there was no significant difference among groups in the mean number of recovered and transferable embryos . In cattle ,the presence of dominant follicle at initiation of gonadotrophin treatment has been also known to decrease the superovulatory response [30-32] , while very few workers did not find any effect [33,34]. It was suggested that the presence of a dominant follicle at the time of initiation of a superovulatory treatment increased atresia of recruitable follicles(4.5-7.9mm) and lowered the ability of follicles >8mm to release estradiol .Hence, presence of a dominant follicle at initiation of gonadotrophin treatment appears

to alter functions of recruitable follicles and their response to superovulatory drug [35,36] In conclusion, the present results showed that the out come of superovulatory response in buffaloes can not be influenced by type of follicular dynamics, moreover the feature of ovarian status at initiation of gonadotrophin treatment tended to be related to superovulatory response was the presence or absence of a dominant follicles.

REFERENCE

1. Misra, A.K., 1997. Application of biotechnologies to buffalo breeding in India.Third Course on Biotechnology of Reproduction in Buffaloes, caserta, Italy, pp: 141-166.

2. Seidel, G.E. and J.R. Seidel, 1991. Training Manual for embryo Transfer in cattle.FAO Animal production and Health Paper 77, FAO, Rome.

3. Misra, A.K., R. Kasiraj, N.S. Mutha Rao, N. Rangae Reddy and B.V. Joshi, 1994. Embryo transfer in buffalo in India: Progress in the last five years. Proc. 4th World Buffalo Congress, 3: 501-504.

4. Aboul-Ela, M.B., 2000. Superovulation in the buffalo, Constraints and Manipulation. Review Article, Buffalo J., 1: 1-20.

5. Taneja, M.G., S.M. Singh, S.M. Totey and A. Ali, 1995. Follicular dynamics in water buffalo superovulated in presence or absence of a dominant follicle. Theriogenol., pp: 581-597.

6. Pierson, R.A. and O.J. Ginther, 1987. Follicular population dynamics during the oestrus cycle of the mare. Anim. Reprod. Sci., 14: 19-231.

7. Fortune, J.E., J. Sirois, A.M. Turzillo and M. Lavoir, 1991. Follicle selection in domestic ruminants. I. Reprod. Fertil. Suppl, 43: 187-198.

8. Taneja, M., A. Ali and G. Singh, 1996. Ovarian follicular dynamics in water buffalo. Theriogenol., 46: 121-130.

9. Baruselli, P.S., R.G. Mucciolo, J.A. Visintin, W.G. Viana, R.P. A rruda, E.H. Madureira, C.A. Oliveira and A. Molero-Filho, 1997. Ovarian follicular dynamics during the oestrus cycle in buffalo(Bubalus bubalis). Theriogenol., 47(8): 1531-1547.

10. Presicce, G.A., A. Parmeggiani, E.M. Sentore, R. Stecco, V.L. Barille, G.J. De Maruo, G. De Santis and G.M. Terzano, 2003. Hormonal dynamics and follicular turnover in prepubertal Mediteranean Italian buffalo. Theriogenol., 8860: 1-9.

11. Danell, B., 1987. Oestrus behaviour, ovarian morphology and cyclical variation in follicular system and endocrine pattern in water buffalo Heifers. Ph.D. Thesis, Uppsala. Swedish University of Agricultural Sciences.

12. Baruselli, P.S., R.G. Mucciolo, R. Amaral, R. Arruda, M.E.O.A. Assumpcao, E.H. Madureira and J.A. Visintin, 1998. Taxa de recuperacao embrionaria em bufalas superovuladas In: Runiao annual da sociedade brasileira De Transferencia De Embrioes, 13, Atibaia, . Arq. Fac. Vet. UFRGS, V. 26, : 22(abst)

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

14. Ginther, O.J., 1993. A method for characterizing ultrasonically-derived follicular data in heifers. Theriogenol., 39: 1-73.

15. Heleil, B., El-S.M. Fattouh, B.H. Serur, S.A. Darwish, and Y.M. El-Deeb, 2009. Superovulation with PMSG (eCG) and embryo Transfer of Egyptian buffalo. Kafrelsheikh Vet. Med. J., 7(2): 174-199.

16. Newcomb, R., W.R. Christie, and L.A. Rowson, 1978. Non surgical recovery of bovine embryo. Vet., Rec., 13: 414-117.

17. Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods. 7th Ed., The Iowa State Univ., Press. Amer. Iowa, USA

18. 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 buffalo *(Bubalus bubalis)* $\geq$ Anim. Reprod. Sci., 92: 241-253.

19. Fortune, J.E., J. Sirois and S.M. Quirk, 1988. The growth and differentiation of ovarian follicles during the bovine oestrus cycle. Theriogenol., 29: 95-109.

20. Azawil, O.I., A.J. Ali. and U.T. Noaman, 2009. A study on the ovarian follicular dynamic in Iraqi Northern buffaloes. Trop. Anim. Health. Prod., 41: 79-83.

21. Warrich, H.M. and N. Ahmed, 2007. Follicular waves during the oestrous cycle in Nilli-Ravi buffaloes undergoing spontaneous and PGF2α induced luteolsis. Anim. Reprod. Sci., 101: 332-337.

22. 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.

23. Terzano, G.M., V.L. Barile, G. De Santis, E.M. Sentore, R. Stecco, G.J. De Maruo, A. Parmggiani and A. Presicce, 2001. Monitoraggio ecografico in buffale Italian sottopost e a sincronizzazione follicolare ed inseminazione strumental. Atti I Congresso Nazionale Sull'allevamento del buffalo. Eboli (sa): 359-362.

24. Fortune, J.E., 1993. Follicular dynamics during the bovine oestrus cycle: A limiting factor in improvement of fertility Anim. Reprod. Sci., 33: 111-125.

25. Adams, G.P., K.K. Kot, C.A. Smith and O.J. Ginther, 1992. Effect of the dominant follicle on regression of its subordinates. Can.J. Anim. Sci., 73: 267-275.

26. Adams, G.P., 1994. Control of ovarian follicular wave dynamics in cattle: implications for synchronization and superstimulation. Theriogenol., 41: 19-24.

27. Nasser, L.F., G.P. Adams, G.A. Bo and R.J. Mapletoft, 1993. Ovarian superstimulstory response relative to follicular wave emergence in heifers. Theriogenol., 40: 713-724.

28. Monniaux, D., D. Chupin, and J. Saumande, 1983. Superovulatory responses of cattle. Theriogenol., 19: 55-81

29. Singh, S.P., P.J. Broadbent, J.S.M. Hutchinson, R.G. Watt. and D.F. Dolman, 1996. Follicular dynamics and superovulatory response in heifer. Anim. Reprod. Sci., 43: 183-190.

30. Rouillier, P., L.A. Guibault, J.G. Lussier, P. Matton, 1996. Changes in morphological appearance and functional capacity of recruited follicles in cows treated with FSH in the presence or absence of a dominant follicle. Theriogenol. 46: 1053-1061.

31. Stock, A.E., J.E. Ellington and J.K. Fortune, 1996. A dominant follicle does not affect follicular recruitment by superovulatory doses of FSH in cattle but can inhibit ovulation. Theriogenol., 45: 1091-1102.

32. Kim, J.H., D.S. Son, S.H. Yeon, S.H. Choi, S.B. Park, I.S. Ryu, G.H. Suh, D.W. Lee, C.S. Lee, H.J. Lee. and J.T. Yoon, 2001. Effect of dominant follicle removal before superstimulation on follicular growth, ovulation and embryo production in holstein cows. Theriogenol., 55: 937-945.

33. Wilson, J.M., A.L. Jones. and D.R. Miller, 1990. Influence of a dominant follicle on the superovulatory response. Theriogenol., 33: 349 (abstr.)

34. Rajamahendran, R. and M.D. Calder, 1993. Superovulatory responses in dairy cows following ovulation of the dominant follicle of the first wave. Theriogenol., 40: 99-109.

35. D'Occio, M.J., D. Jillella and B.R. Lindsey, 1999. Factors that influence follicle recruitment, growth and ovulation during ovarian superstimulation in heifers: Opportunities to increase ovulation rate and embryo recovery by delaying the exposure of follicles to LH. Theriogenol., 51: 9-35.

36. Aerts, J.M.J. and P.E.J. Bols, 2008. Ovarian follicular dynamics. A review with emphasis on the bovine species. Antral development, exogenous influence and future prospects. Reprod. Dom. Anim. doi., 10: 1439-1451.