

Submitted : 05-07-2016

Accepted : 21-08-2016

Published : 15-10-2016

### Ultrasonographic Studies of Follicular Activities in Clomiphene Citrate Treated Anoestrus Cows

Manjusha Patil, M.V. Ingawale, H.S. Birade and and A. Syed Anwar

Department of Animal Reproduction, Gynaecology and Obstetrics

Post Graduate Institute of Veterinary and Animal Sciences, Akola-444 104 (MAFSU)  
Maharashtra

Corresponding Author : [patilmg121@rediffmail.com](mailto:patilmg121@rediffmail.com)

This work is licensed under the Creative Commons Attribution International License (<http://creativecommons.org/licenses/by/4.0/P>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Copyright ©: 2016 by authors and SVSBT.

#### Abstract

An experiment on ultrasonographic studies of follicular activities in clomiphene citrate treated anoestrus cows was conducted. Ultrasound scanning was performed to record the follicular activities on alternate day throughout one estrous cycle for control group and from drug administration to induction of estrus in treatment group. The postpartum induced estrus (treatment group) and regular estrus (control group) differed highly significantly ( $p < 0.01$ ) with respect to number of follicles involved in ovulatory wave ( $6.67 \pm 0.21$  Vs  $5.00 \pm 0.45$ ) and significantly ( $p < 0.05$ ) with respect to growth rate ( $1.82 \pm 0.34$  Vs  $1.04 \pm 0.94$  mm/day) and increase in diameter of dominant follicle ( $7.58 \pm 0.72$  Vs  $10.2 \pm 0.47$  mm). Maximum diameter of ovulatory follicle in induced estrus was greater than regular estrus ( $16.12 \pm 0.56$  Vs  $15.18 \pm 0.47$  mm), however, it differed non-significantly.

**Key Words** : Follicle, Clomiphene, Postpartum regular estrus, Induced estrus, Ultrasonography.

#### Introduction

Follicular growth in cattle is not continuous as initially suggested by studies on ovaries recovered from slaughter houses (Matton *et al.*, 1981) but is cyclic and dependent on the estrous cycle stage (Fortune, 1994). Ovarian follicular dynamics in cows and heifers is characterized by waves of follicular growth and regression during the estrous cycle (Taylor and Rajmahendran, 1991). The real time ultrasonography is a reliable technique for accurate detection of ovarian structures in dairy animals and has gained tremendous popularity in recent years as a diagnostic and research tool in veterinary science. Moreover, accurate timing of the drug administration for induction of estrus is crucial to the success of treatment and it is utmost important to know the size and number of developing follicles prevailing on ovary in induced estrus. This can only be correctly measured by the serial ultrasound technique. Since the hormonal therapy is quite expensive and hence non-hormonal and cost-effective preparations need to be explored for effective treatment of reproductive disorders. Clomiphene citrate, a non-hormonal product has been shown in exerting direct effect on the ovaries of the anoestrus cattle and buffaloes. The status of the follicles at the start of treatment is important for getting better response of Clomiphene citrate. Keeping this in view, the present experiment was planned to study the follicular activities during Clomiphene citrate induced estrus in anoestrus cows.

## Materials and Methods

A total of twelve cows which includes six cows with a history of normal calving and failure to manifest estrus since last three months of calving (Treatment group) and six normally cycling animals which had calved before 3 months (Control group) were used in the study. The animals of the treatment group were treated by intravaginal infusion of 600 mg Clomiphene citrate (2 tabs of 300 mg each; Tab Fertivet®, Ar-Ex Laboratories Pvt. Ltd, Mumbai) by making a suspension in 10 ml of distilled water as one time treatment. The ultrasound scanning was performed on alternate day using a real time B mode ultrasound scanner equipped with a 7.5 MHz convex linear array transducer. The scanning of ovaries was accomplished in several planes to identify all the follicles greater than 4 mm in diameter. Desired images were frozen on the screen and the measurements were recorded. The animals were scanned ultrasonographically on alternate day throughout one estrus cycle for control group and from drug administration to induction of estrus in treatment group.

## Results and Discussion

In control group out of six, five (83.33 %) cows ovulated from dominant follicle from second follicular wave and one ovulated from dominant follicle of third follicular wave. In control group estrus cows ovulated from second dominant follicle (83.33 %) which was similar to that reported by Taylor and Rajmahendran (1991), Filho *et al.* (2001) and Alves *et al.* (2002). On the contrary majority of the cattles ovulated from third dominant follicle (Matton, 1981). In treatment group out of six, four animals (66.67 %) ovulated from dominant follicle of first wave and two failed to ovulate. Number of follicles involved in ovulatory wave was significantly greater in treatment than control group ( $6.67 \pm 0.21$  Vs  $5.00 \pm 0.45$ ; Table 1). In treatment group diameter of ovulatory follicle on day of treatment was  $8.54 \pm 0.39$  mm, while in control group it was  $5.17 \pm 0.09$  mm. The present findings of control group is in agreement with the finding of Ginther *et al.* (1989) in cattle and Manik *et al.* (1998) and Awasthi *et al.* (2006) in buffaloes. However the observed value was greater than the value reported by Kulick *et al.* (1999) for heifers. The emergence diameter of dominant follicle was higher in treatment group than control group.

**Table 1: Comparative ultrasonographic studies of follicular activities in control cyclic group and Clomiphene citrate treated group of anoestrus cows.**

1	No of follicles involved in ovulatory wave	$5.00 \pm 0.45^a$	$6.67 \pm 0.21^b$	3.371**
2	Emergence diameter of dominant follicle (mm)	$5.17 \pm 0.09$	$8.54 \pm 0.39$	1.280 <sup>NS</sup>
3	Maximum diameter of dominant follicle (mm)	$15.18 \pm 0.47$	$16.12 \pm 0.56$	1.280 <sup>NS</sup>
4	Increase in diameter of dominant follicle (mm)	$10.2 \pm 0.47^b$	$7.58 \pm 0.72^a$	2.829*
5	Growth rate of dominant follicle (mm /day)	$1.04 \pm 0.04^a$	$1.82 \pm 0.34^b$	2.834*

\* Significant at  $p < 0.05$ , \*\* Significant at  $p < 0.01$  level, and NS - Non significant.

The follicular waves emerged during a consistent and a narrow time frame of 0-2 days in control group, which is in accordance with Ginther et al. (1989), who concluded that the formation of a follicular wave is well controlled phenomenon that originates in a consistent and narrow time frame. Maximum diameter of ovulatory follicle in treatment group was non-significantly greater than in control group ( $16.12 \pm 0.56$  Vs  $15.18 \pm 0.47$  mm). These findings for diameter of ovulatory follicle in control group are in agreement with the reports of Kulick et al. (1999) in cattle and Awasthi et al. (2006) in buffaloes. Growth rate of ovulatory follicle in cows of treatment group was significantly ( $p < 0.05$ ) greater than control group ( $1.82 \pm 0.34$  Vs  $1.04 \pm 0.04$  mm/day). These findings of growth rate of dominant follicle in control group are in agreement with Kulick et al. (1999) in cattle and Awasthi et al. (2006) in buffaloes. However, the higher daily growth rate in cattle has been reported by Ginther et al. (1989). The higher growth rate of follicle in treatment group may be due to enhanced action of gonadotropins as reported by Manik et al. (1998).

### Conclusion

Better estrus response and growth rate of dominant follicle were observed in Clomiphene citrate treated postpartum anoestrus cows. Ultrasonic imaging is very important and useful technique in the study of patterns of follicular development. Time of onset of estrus could be predicted by the presence of dominant follicle at the time of Clomiphene citrate treatment.

**Conflict of Interest:** All authors declare no conflict of interest.

### References :

- Alves, N.G., da Costa, E.P., Guimaraes, J.D., Silva, M.R., Zamperlini, B., Costa, F.M.J., Santos, A.D.F. and Miranda-Neto, T. (2002). Ovarian Activity in Holstein and Crossbreed Holstein x Zebu Cows During Two Normal Estrous Cycles. *Revista-Brasileira-de-zootecnia*, **31**(2): 627-634.
- Awasthi, M.K., Khare, A., Kavani, F.S., Siddiquee, G.M., Panchal, M.T. and Shah, R.R. (2006). Is one-wave follicular growth during the estrous cycle a usual phenomenon in water buffaloes (*Bubalis bubalis*). *Anim. Reprod. Sci.*, **92**:241-253.
- Filho, S.A.S., Olivera, M.A.L., Caldaa, J.G.L., Lima, P.F. and Donato, I.V. (2001). Ovarian Follicular Dynamics of five-eighths Girolando cows. *Reproduction in Domestic Animals*, **36** (3-4): 207-210.
- Fortune, J.E. (1994). Ovarian Follicular Growth and development in mammals. *Biology of Reproduction*, **50**(2): 225-232.
- Ginther, O.J., Kastelic, J.P. and Knopf, L. (1989). Composition and characteristics of follicular waves during the bovine estrous cycle. *Anim. Reprod. Sci.*, **20**: 187-200.
- Kulick, L.J., Kot, K., Wiltbank, M.C. and Ginther, O.J. (1999). Follicular and hormonal dynamics during the first follicular wave in the heifers. *Theriogenology*, **52**:913-321.
- Manik, R.S., Singla, S.K., Palta, P. and Madan, M.L. (1998). Ovarian follicular dynamics monitored by real-time ultrasonography during estrous cycle in buffaloes (*Bubalus bubalis*). *Asian Australian J. Anim. Sci.*, **11**(5):480-485.
- Matton, P., Adalakoum, V., Couture, Y. and Dufour, J.J. (1981). Growth and replacement of the Bovine Ovarian Follicles during the Estrous cycle. *Journal of Animal Science*, **52**(4):813-820.
- Taylor, C. and Rajamahendran, R. (1991). Follicular dynamics, corpus luteum growth and regression in lactating dairy cattle. *Canadian Journal of Animal Science*, **71**(1):61-68.

□