FOLLICULAR GROWTH, TIME OF OVULATION AND CONCEPTION RATE AFTER SYNCHRONIZATION WITH MEDROXY PROGESTERONE ACETATE IMPREGNATED SPONGES IN NILI RAVI BUFFALO HEIFERS

Muhammad Binyameen[1,*], Saba Anwar[1], Rehana Kauser[2], Azmat Ullah[3], Abdul Rehaman[4] and Mushtaq Ahmad[4]

ABSTRACT

A study was designed to study follicular growth, time of ovulation and conception rate after synchronization with medroxy progesterone acetate sponges in Nili Ravi buffalo heifers. A total of (n=18) nulliparous heifers were selected and divided into two groups on the basis of ultrasonography. Group A (n=9) contained no palpable or visible structure of follicle or Carpus luteum larger than 4 mm, while group B (n=9) contained palpable or visible structure of follicle or Carpus luteum larger than 4 mm on ultrasound screen. The medroxy progesterone acetate impregnated sponge containing 250 mg of progestin was implanted in fornix vagina to each experimental heifer, and day of implantation was declared as D 1. The medroxy progesterone acetate impregnated sponges were implanted up to 6 days. On day 7, sponges were removed with the single injection of pgf2α (+ cloprostenol, 0.075 mg) and GnRH Analog injection (Lecirelin, 50 mcg) was used on day 9 to every experimental animals. Both ovaries were scanned with (Honda 7400, Japan. 7.5 MHz) on D 1, D 6, and D 7 to 9 until ovulation. Ovulation started earlier (23% ovulation at 24 h) and finished later (23% at 36 h) in cyclic heifers as compared to acyclic heifers in which ovulation started 33% at 24 h and finished earlier 56% at 30 h post GnRH Analog injection (Lecirelin, 50 mcg). Estrus response was more pronounced ($P<0.05$) in acyclic heifers as compared to cyclic. Ovulation rate was 100% in cyclic heifers and 89% in acyclic heifers. Conception rate was significantly higher ($P<0.05$) in acyclic heifers (56%) as compared to cyclic heifers (30%). It was concluded from this study that conception rate was higher in cyclic group while ovulation rate was greater in acyclic buffalo heifers' meanwhile, time and size of ovulatory graffian follicle is similar among cyclic and acyclic buffalo heifers in a same synchronization protocol.

Keywords: buffaloes, *Bubalus bubalis*, heifers, sponges, synchronization

INTRODUCTION

Buffalo is a prestigious animal for Pakistani farmers due to its big share in dairy and meat sector. Buffalo reproduction is very sluggish as compared to other dairy animals. Its production is adversely

[1]Buffalo Research Institute, Pattoki District Kasur Pakistan, *E-mail: drbinyameen@yahoo.com
[2]Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan
[3]Livestock and Dairy Development Department, Khyber Pakhtunkhwa, Pakistan
[4]Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan

affected due to higher incidence of reproductive disorders (Akhter *et al*., 2008). Due to lack of homosexual behavior and silent heat, buffalo is different from the cattle (Companile *et al*., 2010; Perera, 2011). However, buffaloes respond well to exogenous hormones and higher conception rate can be achieved by synchronization of ovulation (Rensis and López-Gatius, 2007). Various hormonal protocols have been tried to induce the estrus (Das and Khan, 2010). Prostaglandin alone or with the combination of GnRH satisfactory conception rate can be achieved (Borghese, 2005). To control the estrus cycle different control released products have been used in different animals as reported by (Rathbone *et al*., 1998). For better conception rate in buffalo's different hormonal protocols and their effect on follicular dynamics must be observed (Brito *et al*., 2002). It was first time study in Nili Ravi Buffalo heifers that medroxy progesterone acetate sponges were implanted to check their response on the onset of estrus, changes in growth of follicles, time of ovulation and conception rate.

MATERIALS AND METHODS

Selection of animals

A total of (n=18) buffalo heifers having 400 to 450 kg body weight and age between 36 to 42 months were selected on the basis of ultrasonography (Honda 7400, Japan 7.5 MHz). Both ovaries were scanned twice at the interval of 10 days with transrectal ultrasound for confirmation of ovarian status. Heifers (n=9) bearing follicle more than 4 mm or palpable CL with hand or through transrectal ultrasound were placed in cyclic group. However heifers (n=9) bearing follicle less than 4 mm or lack of palpable CL with hand or through transrectal ultrasound were placed in acyclic group. The feed offered to each experimental buffalo was 40 kg berseem (*Alexendrium Trifolium*), 35 kg corn silage and 5 kg concentrate daily, while water was offered ad libtium. All the experimental animals were tied during the experiment.

Ultrasonography

Both ovaries were scanned through ultrasound (Honda, Japan 7400) having transrectal probe (2.5 M Hz) during selection of experimental animals. Ultrasonography was repeated after 10 days to check any variation in ovarian structure. The heifers having no change in ovarian structure at D 0 and D 10 were placed in (1) Acyclic group (n=9), while animals having different ovarian structure at both time (D 0 and D 10) during ultrasonography were placed in (2) Cyclic group (n=9. Furthermore, follicular dynamic of both ovaries was scanned after every 6 h from day 7 to 9 until ovulation. Absence of graffian follicle having a size of 9 mm or above was confirmatory sign of ovulation.

Treatment

Medroxy progesterone sponges containing 250 mg of Medroxy progestin were prepared by Robinson's method (Robinson, 1965) and implanted in all experimental animals. The day of implantation was declared as D 1. On D 7 sponges were removed with a single injection of pgf2α (+ cloprostenol, 0.075 mg). After 48 h of first injection, GnRH Analog injection (Lecirelin, 50 mcg) was administered to each experimental animal.

RESULTS AND DISCUSSION

Ultrasonography was carried out after every six hour post GnRH Analog injection (Lecirelin, 50 mcg) until ovulation. There was clear mucus

discharge, swollen valve when size of graffian follicle exceeded from 11 mm and estrus response was maximum 60 h after removal of Medroxy progesterone acetate sponges. The size of graffian follicle and time of ovulation at different time intervals post GnRH Analog injection (Lecirelin, 50 mcg) in cyclic and acyclic buffalo heifers is shown in Table 1 and Table 2 respectively.

The estrus response was maximum 60 h after the removal of Medroxy progesterone Acetate sponges with the presence of graffian follicle more than 11 mm and clear mucous discharge were positive sign of estrus. One heifer failed to ovulate in acyclic group as shown in Table 2. Average time

Table 1. Size of graffian follicle (mm) post GnRH injection at different time intervals in cyclic buffalo heifers until ovulation.

Serial #	0 h	6 h	12 h	18 h	24 h	30 h	36 h
1	6.5	10.5	13.6	14.3	Ovulation	-	-
2	4.5	6.5	9.7	11.2	13.5	ovulation	-
3	4.7	6.2	8.1	9.5	12.5	14.3	Ovulation
4	3	3	5.3	8	11.3	11.3	Ovulation
5	4.4	7.5	9.2	11	14.2	ovulation	-
6	3.9	7	10.5	12.5	ovulation	-	-
7	6.9	11.2	13.5	15.2	ovulation	-	-
8	5.3	8.7	11	12.5	14.6	Ovulation	-
9	4.1	7.3	13.5	15.6	Ovulation	-	-

Table 2. Size of graffian follicle (mm) post GnRH injection at different time intervals in acyclic buffalo heifers until ovulation.

Serial #	0 h	6 h	12 h	18 h	24 h	30 h	36 h
1	4.3	5.5	8.5	14.3	15.4	ovulation	-
2	6.8	11	12.2	15.2	Ovulation	-	-
3	3.9	4.3	6.5	6.5	6.5	6.5	Unovulated
4	3.7	6.5	8.8	8.8	8.8	ovulation	-
5	4	6.6	9.4	9.4	ovulation	-	-
6	5.1	7.8	9	11.4	14.6	ovulation	-
7	4.9	7.3	10.5	14.3	Ovulation	-	-
8	6.1	7.6	9.5	12.6	14.4	ovulation	-
9	4.7	6.9	9.7	12.5	15.3	ovulation	-

of ovulation (hours) post GnRH injection in cyclic and acyclic buffalo heifers until ovulation was same ($P<0.05$) in both group. Ovulation started earlier (23% ovulation at 24 h) and finished later (23% at 36 h) in cyclic heifers as compared to acyclic heifers in which ovulation started 33% at 24 h and finished earlier 56% at 30 h post GnRH Analog injection (Lecirelin, 50 mcg). Our results are in line with (Paul and Parkash, 2005) who reported ovulation occurred earlier and lasted for longer time in cyclic animals as compared to acyclic buffalo heifers. Our results are also similar to (Ali and Fahmy, 2007) and (Karen and Darwish, 2010) who reported early ovulation in acyclic buffalo's heifers as compared to cyclic buffaloes. Our results are in line to (Perry *et al*., 2007) who reported that maximum estrus response observed when follicle size reached between 11 to 12 mm. The maximum size of follicles at the time of ovulation reported by (Sharma *et al*., 2012) was 16.07±0.99 mm which is more close to our study. The result of this study are similar to (Mirmahmoudi *et al*., 2014) who gave the range of 11 to 15 mm size of follicles at the time of ovulation. This study is opposite to (Sartori *et al*., 2001) who reported that follicle less than 10 mm are unable to ovulate in cows. This difference might be variation from species to species and parity.

CONCLUSION

It was concluded from this study that conception rate was higher in cyclic group while ovulation rate was greater in acyclic buffalo heifers'. The ovulation time and size of ovulatory follicle was similar among cyclic and acyclic buffalo heifers in a same synchronization protocol. All heifers expressed pronounced estrus response when size of graffian follicle exceeded from 11 mm.

REFERENCES

Akhtar, S.M., L.A. Lodhi., I. Ahmad, Z.I. Qureshi and G. Muhammad. 2008. Serum concentrations of calcium, phosphorus and magnesium inpregnant nili-ravi buffaloes with or without vaginal prolapse in irrigated and rain fed areas of Punjab. *Pak. Vet. J.*, **28**(3): 107-110.

Ali, A. and S. Fahmy. 2007. Ovarian dynamics and milk progesterone concentrations in cycling and non-cycling buffalo-cows (*Bubalus bubalis*) during Ovsynch program. *Theriogenology*, **68**(1): 23-28.

Borghese, A. 2005. *Technical Series 67*. Food and Agriculture Organization, Rome, Italy.

Brito, L.F.C., R. Satrapa, E.P. Marson and J.P. Kastelic. 2002. Efficacy of PGF2α to synchronize estrus in water buffalo cows (*Bubalus bubalis*) is dependent on plasma progestrone concentration, corpous leutum size and overian follicular statusbefore treatment. *Anim. Reprod. Sci*., **73**: 23-35.

Campanile, G., P.S. Baruselli, G. Neglia, E.M. Senatore, A. Bella, G.A. Presicce and L. Zicarelli. 2011. Pregnancy rate following AI with sexed semen in Mediterranean Italian buffalo heifers. *Theriogenology*, **76**: 500-506.

Das, G.K. and F.A. Khan. 2010. Summer anoestrus in buffalo-A review. *Reprod. Domest. Anim.*, **45**(6): e483-e494.

Karen, A.M. and S.A. Darwish. 2010. Efficacy of Ovsynch protocol in cyclic and acyclic Egyptian buffaloes in summer. *Anim.*

Reprod. Sci., **119**(1-2): 17-23.

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**(2): 237-242.

Paul, V. and B.S. Prakash. 2005. Efficacy of the Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes (*Bubalus bubalis*). *Theriogenology*, **64**(5): 1049-1060.

Perera, B.M. 2011. Reproductive cycles of buffalo. *Anim. Reprod. Sci*., **124**(3-4): 194-199.

Perry, G.A., M.F. Smith, A.J. Roberts, M.D. MacNeil and T.W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J. Anim Sci*., **85**: 684-689.

Rathbone, M.J., K.L. Macmillan, W. Jochle, M. P. Boland and E.K. Inskeep. 1998. Controlled-release products for the control of the estrous cycle in cattle, sheep, goats, deer, pigs and horses. *Crit. Rev. Ther. Drug*., **15**: 285-380.

Rensis, F.De. and F. López-Gatius . 2007. Protocols for synchronizing estrus and ovulation in buffalo (*Bubalus bubalis*): A review. *Theriogenology*, **67**(2): 209-216.

Robinson, T.J. 1965. Use of progestagen-impregnated sponges inserted intravaginally or subcutaneously for the control of the oestrous cycle in the sheep. *Nature*, **206**: 39-41.

Sartori, R., P.M. Fricke, J.C.P. Ferreira, O.J. Ginther and M.C. Wiltbank. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicle. *Biol. Reprod*., **65**(5): 1403-1409.

Sharma, R.K, J.K. Singh, S. Khanna and I. Singh. 2012. Ovarian response of prepubertal Murrah heifers to exogenous GnRH. *Anim. Reprod. Sci*., **133**(3-4): 153-158.