

Effect of GnRH and PGF₂ on uterine involution and post-partum fertility in crossbred cows

SURESH KUMAR¹, RAMESH CHANDRA², NAZRUL HAQUE³, SAROJ TOPPO⁴ and H RAHMAN⁵

ICAR Research Complex for NEH Region, Sikkim Centre, Tadong, Gangtok 737 102

Received: 6 December 2009; Accepted: 21 September: 2010

ABSTRACT

The present experiment was conducted in recently parturited crossbred cows to study the effect of GnRH and prostaglandin administration on uterine involution and resumption of ovarian cyclicity. The animals in group 1 (n=9) were administered GnRH (buserlin acetate, 20 µg, I/M) on day-16 post-partum, followed by PGF₂ (tiaprost 725 µg) on day 24 post-partum and second group animals (n=6) were administered same amount of NSS as placebo. The animals in group 1 showed significant improvement in uterine involution (33.3±2.66 vs 42.66±5.89 days) and resumption of post-partum cyclicity (36.22±5.89 vs 57.16±8.36 days) as compared to control. Hence it can be concluded from the present study that administration of GnRH-PGF₂ in early post-partum period hastens the uterine involution and improves the reproductive efficiency of crossbred cows.

Key words: Fertility, Involution, Post-partum, Uterus

The normal process of uterine involution is an aseptic process in which various cells originating from maternal placenta, inter-coruncular mucosa and blood are shred and degenerated. Uterine involution is concluded around 45 d postpartum and consists of the reduction in size, loss of tissue, and tissue regeneration of the uterus (Kiracofe 1980). Reduction in size is, in general, a response of myometrial contractility that plays a major role in clearing lochial debris from the uterus after calving (Slama *et al.* 1991, Hirsbrunner *et al.* 2002).

The process is exceedingly rapid in the initial stages. There is rapid and progressive loss of collagen in the muscle cell cytoplasm and ground substances produced. Puerperal uterine soundness is essential for the establishment of postpartum estrus cyclicity. Retention of fetal membranes is one of the common melodies during the puerperium in cows and buffaloes and its effect on subsequent fertility is important. Delayed involution, chronic metritis and poor conception rate are its common sequelae. In order to obtain maximum economic gain, the animals must conceive again at the optimum time after parturition, This is only possible when the reproductive organs are clean enough and also have

regained its functionality again to be capable enough for a successful reproduction. The involution of uterus and resumption of ovarian activity are the important rebound events which are prerequisite for next successful conception. Endocrinological responses resulting into release of PGF₂ and GnRH help in regaining the post-partum reproductive activity. The dinoprost, tiaprost and other analogues have been used to hasten the puerperium with early post-partum estrus (Sinha *et al.* 2002, Sheshappa *et al.* 2002). Cows with a short period of high PG metabolites required relatively longer period for complete uterine involution as PGF₂ has a positive effect on the uterine muscular tone. Hence the present study was conducted to establish the chronology of events of uterine involution with respect to location, size and consistency in a physiological time frame and effect of GnRH and PGF₂ on uterine involution, resumption of post-partum ovarian activity and conception rate in crossbred cattle.

MATERIALS AND METHODS

The study was conducted on normally parturited crossbred cows from first to fourth lactation maintained under identical standard feeding and managerial but stalled condition at Dairy Research Unit of ICAR Research Complex for NEH Region, Sikkim Centre, Tadong, Gangtok. The animals were randomly divided into 2 groups. In group 1, the animals (n=9) were administered GnRH (acetate 20µg I/M) on day 16 post-partum and followed by PGF₂ (750µl/M) on day 24 post-

Present address: ¹Principal Scientist (Animal Reproduction), ICAR Research Complex for NEH Region, Umiam, Meghalaya (e mail: suresh_vet079@redifmail.com).

³Principal Scientist (Animal Nutrition), NRC on Mithun, ICAR, Jharnapani Medziphema, Nagaland.

^{2,4}Senior Scientist, ⁵Joint Director.

partum called the treatment group (T) whereas in group 2 the animals (n=6) were given 5 ml of NSS on day 16 and day 24 post-partum placebo, I/M served as control (C). All the animals were examined per-rectally on day 8, 16, 24 and every alternate day thereafter till completion of involution of uterus. Uterine score was a combination of size and tonicity of the uterus assessed by palpation per rectum. Dynamics of the size of the uterus obtained by palpation per rectum during postpartum period was conducted based on Zemjanis (1970). Complete involution was judged on rectal palpation by (i) return of uterus to its normal location in the pelvic cavity i.e intrapelvic or near pelvic brim; (ii) normal and approximately equal size of uterine horns; and (iii) attainment of normal uterine texture, tone and consistency.

On each per rectal examination the observations regarding the location of the uterine horns, size of uterine horns and cervix, consistency and tonicity of uterine musculature, degree of reduction in size of gravid and non gravid uterine horns, exhibition of ovarian activity were recorded. In addition exhibition of estrus signs and fertile estrus/conception rate within 100 days post-partum were also recorded and followed-up. The data were analysed using student's t test as per Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The results indicated that in all the animals the uterus and the cervix both were hanging below the pelvic symphysis and located in the abdominal cavity on day 8 post-partum while by day 16, about 60% (09/15) of animals had their genitalia returned to the pelvic cavity. Remaining 40% (06/15) animals still had their genitalia hanging either over the pelvic symphysis or in the abdominal cavity. By day 24 all the animals had their genitalia totally in the pelvic cavity except 2 animals from the control group whose genitalia were partially hanging over the pelvic symphysis and both of them were pluriparous.

On day-8 the tip of non gravid horn could be palpated easily but the tip of the gravid horn could not be palpated efficiently, however, on day-16, tip of both the horns could be palpated easily. The size of the individual uterine horn was larger in diameter than the diameter of cervix on day 16. The diameter of individual uterine horn was almost similar to the diameter of cervix by day 24. The rate of reduction of

size of uterine horns was much faster in first 2 weeks, which decreased gradually thereafter. By the end of fourth week the diameter of the cervix exceeded the diameter of individual uterine horn of all the animals in the treatment group. In the control group, however, only 33% (02/06) cows had higher cervical diameter than diameter of individual horn. The non-gravid horns showed apparently almost normal size in both the groups by day 24–28 post-partum. The reduction in size of gravid uterine horn was faster in treated group than the control during the fourth and fifth week.

The regular per-rectal examinations revealed that complete involution of uterus took significantly ($P<0.01$) less time (33.33 ± 2.66 days) in treated group as compared to control group (42.66 ± 5.89 days) and reduced the period of involution by almost 09 days (Table 1). The spasmogenic effect of $\text{PGF}_2\alpha$ on the uterine musculature has been established (Rodríguez-Martínez *et al.* 1987). Our findings corroborates with the findings of Albuquerque (1986) who reported that the time taken for the complete involution in different treatment groups was lower in comparison to control group. Uterine involution is dependent upon the concentration of PG metabolites at parturition, status of micro flora present (Deka *et al.* 1985) and is generally delayed with increased parity (Peter *et al.* 1987) and during colder season (Etherington *et al.* 1985).

To our knowledge, uterine tone has been barely evaluated as an outcome variable in clinical trials conducted on dairy cattle. Reasons for this are the subjectivity of the method. However, using a well-defined scoring system, which is conducted consistently, usefulness of uterine tonicity might have practical implications (Loeffler *et al.* 1999). Indeed, in studies evaluating ovarian anovulatory conditions (ovarian cysts), uterine tonicity has been a key factor for diagnostic purposes (Bartolome *et al.* 2000). The difference in uterine tonicity and size might be a reflection of uterine contractility, reduction of uterine diameter, and shorter time until anatomical involution after $\text{PGF}_2\alpha$ treatment. Uterine tone and diameter of the uterine horns have been related in the early postpartum period. Cows with smaller uterine diameters had greater tonicity (Slama *et al.* 1991).

Similarly, the resumption of post-partum ovarian cyclicity as confirmed by per-rectal examination and also aided by exhibition of discharge and/or estrus signs (in some cases)

Table 1. Effect of GnRH and $\text{PGF}_2\alpha$ on involution of uterus and post-partum fertility in crossbred cows

Groups	No. of animals	Uterine involution (days)	Resumption of PP ovarian activity (days)	No. of animals conceived (within 100 days pp)	Conception rate (%)
Treatment group (GnRH+ $\text{PGF}_2\alpha$)	09	$33.33\pm2.66^{**}$	$36.22\pm7.53^{**}$	06/09	6.6 ^{**}
Control group	06	42.66 ± 5.89	57.16 ± 8.36	02/06	33.33

^{**}Indicates highly significant ($P<0.01$).

took a period of 36.22±7.53 days in treated group as compared to 57.16±8.36 days in control group significantly ($P<0.01$) shorter by about 21 days or almost one complete cycle indicating the beneficial effect of GnRH and PGF₂ in cleansing the uterus and resumption of ovarian cyclicity post-partum. The observations based on conception rate within 100 day post-partum revealed that the treated group had a conception rate of 60% (06/09) as compared to 33.33% (02/06) in the control group.

First postpartum and fertile estrous have been reported to vary with gestation length, status of micro flora in the uterus (Deka *et al.* 1985), parity (peter *et al.* 1987) and season (Etherington *et al.* 1985). Kumar and Purbey (1987) observed that in rural bovines first estrus appeared at 9.7±0.98 months after calving. McClary *et al.* (1989) and Epsana *et al.* (1992) also suggested that there was a decline in time interval for first postpartum estrus after administration of PGF₂ or its analogue. However, Albuquerque (1986) and Pinheiro *et al.* (1990) did not find any change in these parameters. Melendez *et al.* (2004) observed that 2 doses of PGF₂, 8 h apart at 8 d post-partum in primiparous cows with acute puerperal metritis decreased the diameter of uterine horns and serum concentration of β 1- acid glycoprotein at 12 d postpartum and increased the conception rate at first service. These observations support our findings.

The time taken for resumption of the ovarian activity following parturition regulates the calving interval. The GnRH administration on day 14 post-partum stimulated the reproductive physiology and assisted in the cleansing of uterus and bringing faster uterine involution thereby improving the fertility and conception rate (Foote and Riek 1999 and Takkar *et al.* 1999). Further, prostaglandin F₂ and its analogues with their most potent luteolytic activity brings revolutionary advancement in regulation of the estrous cycle of dairy animals (Okuda *et al.* 1988, Epsana *et al.* 1992).

The mean duration or complete uterine involution, exhibition of first post-partum estrus and conception rate are presented in the Table 1. The beneficial effect of GnRH therapy on day 14 post-partum in terms of shorter duration of uterine involution observed in the animals of treated group of the present study is in agreement with the findings of earlier workers in cows (Foote and Riek 1999) and in buffaloes (Honnappagol 1991, Takkar *et al.* 1999). This can be attributed to the fact that responsiveness of endogenous LH to exogenous GnRH increased during day 10–18 post-partum with a resultant decrease in calving interval, lesser number of services per conception and high conception rate in post-partm cows. In the animals where a combined therapy of GnRH and PGF₂ was employed had shorter uterine involution interval compared to animals where GnRH and PGF₂ was employed had shorter uterine involution interval compared to animals where GnRH was given alone on day 14 post-partum (Sheshappa *et al.* 2002) suggesting the additional advantage of PGF₂ in association with GnRH.

This is in agreement of earlier report of Okuda *et al.* (1988). This observation is supported by the fact that a positive myometrial effect of PGF₂ appears to promote early luteolytic activity following the exogenous administration in cows.

Hence it can be concluded from the present investigation that in healthy normal crossbred cows the genitalia returns to the pelvic cavity by day 24 post-partum and complete gross involution of uterus takes about 42 days and the ovarian activity resumes by day 58. Incorporation of GnRH - PGF₂ regimen significantly hastenst the uterine involution as well as resumption of post-partum activity and also improves the post-partum fertility.

ACKNOWLEDGEMENT

The authors are highly thankful to Director ICAR Research Complex for NEH Region, Umroi Road, Umiam, Meghalaya for providing necessary facilities.

REFERENCES

- Albuquerque F T. 1986. Effect of cloprostenol on the time required for uterine involution in Holstein Friesian $\times$ zebu crossed cows. *Arquivo Brasileiro de Medicina Veterinaria Zootecina* **8**: 769–98.
- Bartolome J A, Archbald A L F, Morresey P, Hernandez J, Tran T, Kelbert D, Long K, Risco C A and Thatcher W W. 2000. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies or bovine ovarian cysts in the dairy cow. *Theriogenology* **53**: 815–25.
- Deka K G, Nath K C and Rajkonwar K C. 1985. Postpartum changes of uterus and ovaries in relation to uterine microflora in cows. *Indian Journal of Animal Reproduction* **6**: 122.
- Espana Espana F, Vivo Ro Driwez R and Lopez Schostain A. 1992. GnRH and PGF₂ alpha hormone treatment during the puerperium in dairy cattle. Effect on length of the reproduction cycle. *Archivis de Zootecnia* **41**: 65–73.
- Etherington W C, Marin S W and Dohoo I R. 1985. Interrelationship between ambient temperature, age at calving post-partum reproductive performance in dairy cows: a path analysis. *Canadian Journal of Complementry Medicine* **49**: 254–60.
- Foote R H and Riek P M. 1999. Gonadotropin releasing Hormone improves reproductive performance of dairy cows with slow involution of the reproductive tract. *Journal of Animal Sciences* **77**(12): 12–16.
- Hirsbrunner G B, Knutti I, Liu U, Küpfer G, Scholtysik and Steiner A. 2002. An *in vivo* study on spontaneous myometrial contractility in the cow during estrus and diestrus. *Animal Reproduction Science* **70**: 171–80.
- Honnappagol S S. 1991. 'Studies on post-partum anestrus in Surti buffaloes with reference to clinico- biochemical and hormonal status'. Ph.D. Thesis submitted to Deemed University Indian Veterinary Research Institute Izatnagar UP, India.
- Kiracofe G H. 1980. Uterine involution: Its role in regulating postpartum intervals. *Journal of Animal Science* **51** (Suppl. 2): 16–28.
- Kumar S and Purbey L N. 1987. Postpartum fertile estrus interval in rural bovines. *Indian Journal of Animal Reproduction* **8**: 112–15.
- Loeffler S H, de Vries M J, Schukken Y H, de Zeeuw A C,

- Dijkhuizen A A, de Graaf F M and Brand A. 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Theriogenology* **51**: 1267–84.
- McClary D G, Putnam M R, Wright J C and Sartin J L Jr. 1989. Effect of early postpartum treatment the PGF₂ alpha on subsequent fertility in dairy cows. *Theriogenology* **31**: 565–70.
- Melendez P, McHale J, Bartolome J, Archbald L F and Donovan G A. 2004. Uterine involution and fertility of holstein cows subsequent to early postpartum PGF₂ treatment for acute puerperal metritis. *Journal of Dairy Science* **87**: 3238–46.
- Okuda K, Gaona W A and Sato K. 1988. Effect of gonadotropin – releasing hormone and prostaglandin F₂ on the reproductive performance in post-partum cows. *Theriogenology* **29**(4): 823–33.
- Peter A T, Narshima K S John D and Patabiraman S R. 1987. Studies on involution of uterus in postpartum Murrah buffaloes. *Indian Journal of Animal Reproduction* **8**: 1–3.
- Pinheirn I E I, Guimaacs J D and Crathora M. 1990. Effect of PGF₂ alpha treatment during the early postpartum period. *Brasibira de Repeoducacao Animal* **14**: 65–71.
- Rodrigues- Martincz H, Ko J, McEnna D C, Weston P G, Whitmore H L, Gustafsson N B K and Wagner W C. 1987. Uterine motility in the cow during the estrus cycle ii. Comparative effect of PGF₂ alpha, E₂ and Cloprostenol. *Theriogenology* **27**: 349–358.
- Sheshappa H, Honnappagl S S, Dhoble R L and Tandle M K. 2002. Efficacy of GnRH and PGF₂ in augmenting the reproductive performance of post partum cows. *Indian Journal of Animal Reproduction* **23** (1): 75–76.
- Sinha V K, Singh Balraj and Sinha A K. 2002. Management of postpartum reproduction in cross bred cows with dinoprost. *Indian Journal of Animal Reproduction* **23** (1): 21–24.
- Slama H, Vaillancourt D and Goff A K. 1991. Pathophysiology of the puerperal period: Relationship between prostaglandin E₂ (PGE₂) and uterine involution in the cow. *Theriogenology* **36**: 1071–90.
- Snedecor G W and Cochran W G. 1980. *Statistical Methods*. 7th edn, Pp.32–62. Oxford and IBH Publishing company, 17, Park Street, Calcuta.
- Takkar O P, Singh Narinder, Kaur Jagjit and Chaudhary K C. 1999. Augmentation of post-partum reproductive efficiency by the use of GnRh in buffaloes. *Indian Journal of Animal Reproduction* **20** (1): 9–11.
- Zemjanis R. 1970. Examination of the cow. *Diagnostic and Therapeutic Techniques in Animal Reproduction*. 2nd edn, pp 3–87. (Ed.) Zemjanis R. The Williams & Wilkins Company, Waverly Press, Inc., Baltimore, MD.