

Reproductive Performance of Cross-bred Sudanese Dairy Cows Treated with GnRH During Early Postpartum

F. O. A. Elzubeir and A. S. Elsheikh

Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine,
University of Khartoum, Shambat, Sudan

Abstract: The present study was designed to investigate the effects of GnRH injection during early postpartum on uterine involution, length of postpartum period, number of services per conception, days open and calving interval in cross-bred Sudanese dairy cows. A total of 20 cross-bred dairy cows were used to examine the effects of GnRH on the above mentioned parameters. The cows were grouped into 2 groups: A (n = 15) and B (n = 5). Group A was further grouped into 3 subgroups (5 cows each). The first group was given a single injection of GnRH (200 μ g) i.m on day 7, the second and third groups were given the same dose of GnRH on day 14 and day 21, respectively. Cows in group B were employed as untreated control. The results of the present study showed that, injection of GnRH during the third week postpartum has no effect on uterine involution, whereas, injection of GnRH during the first or the second week postpartum significantly reduced ($P < 0.005$ and $P < 0.05$, respectively) the time taken for uterine involution, compared to the control. The postpartum period of the cows treated with GnRH as early as first, second or the third week postpartum was significantly ($P < 0.05$) shorter as compared to the control. The shortest postpartum period was achieved by injecting GnRH during the third week. The cows received an injection of GnRH during the first, second or the third week postpartum has a similar number of services per conception (1.00 ± 0.00) which was significantly different ($P < 0.04$) from that of the control (1.40 ± 0.20). Moreover, their days open and calving interval were significantly reduced ($P < 0.001$) compared to that of the control. It is concluded that the use of GnRH during early postpartum improves the reproductive efficiency of cross-bred dairy cows.

Key words: Reproductive, cross bred, sudanese, dairy cows, GnRH, postpartum

Introduction

The long interval from parturition to the recrudescence of ovarian cyclicity is a major problem that limits the improvement of reproductive efficiency of dairy cows reared under tropical conditions (Short *et al.*, 1990 and Williams, 1990). The cross-bred dairy cows in the Sudan are known to have a long postpartum intervals to first oestrus, 162.90 ± 2.70 days (Elhag, 2003), which affects their reproductive efficiency. The prolonged postpartum acyclicity is attributed to the absence of appropriate LH pulses, which lead to atresia of follicles develop postpartum (Yavas and Walton, 2000). During early postpartum, pituitary LH stores are depleted (Nett, 1987 and Nett *et al.*, 1987). Around the third week postpartum LH stores replenished (Yavas and Walton, 2000). The same authors reported that, the initiation of postpartum ovarian cyclicity could be induced by injection of GnRH during early postpartum to increase LH pulse frequency. GnRH is a potential drug that is used widely in the management of postpartum reproductive efficiency in dairy cows (Fernandez *et al.*, 1978). Administration of GnRH to dairy cows reduces the period of first postpartum oestrus (Thatcher *et al.*, 1993). Twagiramungu *et al.* (1995) showed that administration of GnRH eliminates large follicles by ovulation or atresia and initiates new follicular waves within 3 to 4 days after treatment at any stage of oestrous cycle. However, it limits further growth of these emerging follicles by increasing atresia. Cows given GnRH on day zero postpartum were reported to show oestrus on day 10 (Mialot *et al.* 1999). Also, the same authors reported that the pregnancy rate reached 53.7% for the cows injected with GnRH on the day of calving. Injection of GnRH two weeks postpartum induced ovulation, cyclic activity, shortened the calving interval and improved conception rate in none suckling dairy cows (Fernandez *et al.*, 1978; Lamming *et al.*, 1982 and Risco *et al.*, 1995). Contrary, the treatment of cows with GnRH had no effects on the first oestrous cycle postpartum, on services per conception, days opens, or any other reproductive trait measured (Foote and Riek, 1999). Cows given GnRH two weeks postpartum experienced an improved rate of uterine involution, short calving intervals and a better rate of conception to first service in non suckling dairy buffaloes (Nasir, 1990; Guilbault *et al.*, 1985). Dairy cows given GnRH two weeks postpartum undergone slow involution of the reproductive system with no difference from the control (Leslie *et al.*, 1984). In the view of the above mentioned findings, the present study was designed to investigate the effects of GnRH injection during early postpartum on uterine involution, length of postpartum period, number of services per conception, days open and calving interval in cross-bred Sudanese dairy cows.

Materials and Methods

Study Area: This study was conducted at Khartoum University Dairy Farm. It lies within the semi-arid zone at

latitude 15:16° and longitude 32:32°, 376 meters above sea level. The average rainfall per year is 167 mm. The maximum rainfall is reached between July and September. Temperature is very high and it exceeds 45°C during the days of summer months (March to June).

Animals: Twenty multiparous cross-bred dairy cows (Friesian × Kenana) were employed in this study. Their body condition scores (BCS) vary between (2.5 to 3.5) according to the scale based on five points out lined by Wildman *et al.* (1982). According to this scale emaciated cows are scored 1, thin cows 2, average cows 3, fat cows 4 and obese cows 5. The life weights at delivery were between (200 to 400 kg). The cows employed for the purpose of this study, calved during 2002. Cows were milked twice a day in the morning and evening. All animals were fed roughages ad-libitum as group but were individually fed dairy concentrate twice at milking time (10 kg/cow). The concentrate consist of 37% sorghum, 21% cotton seed cake, 40% wheat bran and 2% sodium chloride. Throughout the experimental period, the cows were allowed to graze daily from 7:00 a.m. to 9:00 a.m. on *Vicia faba* (broad beans) and *Sorghum bicolor* (Abu 70) residues. The roughage offered to the cows in the pens during the day consisted of green Alfa alfa (barseem) and Abu 70. After parturition, the new-born calves were separated from their dams and were bucket fed.

Routine testing for brucellosis and vaccination against the major diseases were practiced, once every year. The cows were drenched with Elbendazol at a dose of 3 ml/10 kg body weight (Benvet suspension, 2.5% USP, CIPLA LTD, Mumbai 400 INDIA) to control internal parasites. Spraying of acaricides for both animals and sheds is frequently done to control external parasites.

The experimental animals were kept in open shaded yards constructed with iron bars, partially roofed with metal sheets and the floor was covered with sands. Animals were grouped according to age and level of production.

Milk Sampling: Milk samples were collected every week following parturition till the first observed heat. The samples were collected from evening milking using 20 ml clean plastic vials. Then a tablet of sodium azide (100 mg/10 ml) was added to the whole milk as preservative and immediately the samples were centrifuged for 15 minutes at 2,500 g for the skimming of fat (Labofuge, 200, Kendro Laboratory products, Germany). Just after that, the milk samples were placed in a refrigerator (4°C) for 15 minutes to harden the fat layer. Then a glass rod was used to pierce the fat layer. Then the entire skim milk samples were transferred in 4.5 ml cryogenic vials (Nalgene®, Nalge Company. Rochester, NY 14602-6365, lot 366947 USA) using Pasteur pipette (23cm length) and pipette bulbs and stored at -20°C until assayed for progesterone concentrations.

Serum Progesterone Radioimmunoassay (RIA): The progesterone concentration in this study was determined according to the FAO/IAEA protocol for progesterone RIA (1996). The detecting limit of this assay is 0.02mg/ml.

Uterine Involution: Uterine involution was determined by rectal palpation every other day after parturition till complete involution. The uterine involution was considered complete when the uterine horns returned to the position, size and tone of a non pregnant or cyclic animal (Casida *et al.*, 1968 and Arthur *et al.*, 1998). The complete uterine endothelium involution was assumed when the animal express the first postpartum heat signs.

Heat Detection: Well-trained herdsmen observed all the animals thrice daily for heat symptoms. The animals were observed for duration of 20 minutes at 6:00 am, 2:00 p.m and 10:00 p.m. The cow was considered in oestrus when it stand to be mounted by other cows, mounted others, a clear vaginal mucous discharge hanged from its vulva and when it bellowed. The manifestations of these symptoms varied from one cow to another but fortunately the herd men were able to detect heat. The occurrence of postpartum oestrus was confirmed by progesterone RIA (Gong *et al.*, 2002).

Cow Service: Cows exhibited oestrous behaviours in a period less than 42 days postpartum were not served. But those, which exhibited oestrous behaviour after that time, were served with a cross-bred bull with a proven fertility. Number of services per conception had been calculated according to Arthur *et al.* (1998) from the number of services given to the experimental animals after recrudescence of the postpartum oestrus and resulted in diagnosed pregnancy not less than 42 days after service.

Pregnancy Diagnosis and Days Open: Pregnancy diagnosis was carried out for none return cows by rectal palpation at 45 days after last service (Arthur *et al.*, 1998). Days open had been determined by counting the interval, in days, from calving to the subsequent effective service date for those cows that conceived (Arthur *et al.*, 1998).

Calving Interval: Calving interval was calculated according to Arthur *et al.* (1998) and Bath *et al.* (1985) where the calving interval is the period between two consecutive calvings.

Experimental Design: This experiment was designed to study the effects of GnRH treatment on uterine involution, recrudescence of the first postpartum oestrous (length of postpartum period), length of the open period, number of services per conception and calving interval in crossbred Sudanese dairy cows. A total of 20 cross-bred dairy cows were used to examine the effects of GnRH on the above mentioned parameters. The cows were randomly divided into 2 groups A ($n = 15$) and B ($n = 5$). Group A was further sub grouped into 3 subgroups (5 cows each). The first subgroup was injected (i.m) with 200 μ g synthetic GnRH (Fertagyl 0.10 mg/ml, Intervet, lot 20023 A, Boxmeer, Holland) on day 7 postpartum (Nasir *et al.*, 1990). The second subgroups were injected with the same dose of Fertagyl on day 14 and day 21 postpartum, respectively. Group B was employed as untreated control. Uterine involution, heat detection, the number of service per conception, days open and calving interval were assessed as mentioned in the materials and methods. Milk sampling was collected as mentioned in the materials and methods.

Statistical Analysis: Data are means \pm standard error of the mean (SEM). The results were statistically evaluated by ANOVA followed by Fisher's protect least significant difference (PLSD). Differences at probability of $P < 0.05$ were considered to be statistically significant.

Results

The results of the present study showed that, a single injection of GnRH during the third week postpartum has no effect on uterine involution, whereas, injection of GnRH during the first or the second week postpartum significantly ($P < 0.005$ and $P < 0.05$, respectively) reduced the time taken for uterine involution, compared to the control. The mean length of the time taken for uterine involution in cows received a single injection of GnRH during the first or the second week postpartum were 15.40 ± 0.70 and 19.00 ± 0.40 days respectively. These values were significantly lower than that of the cows received a single injection of GnRH during the third week postpartum and control (27.40 ± 0.90 days and 27.20 ± 1.00 days, respectively) (Fig. 1).

The postpartum period of the dairy cows that received a single injection of GnRH as early as first, second or the third week postpartum was significantly ($P < 0.05$) shorter as compared to the control. However, injection of GnRH during the third week was better than injection during the second week ($P < 0.05$) or the first week ($P < 0.001$). Furthermore, the injection of GnRH during the second week was better than injection during the first week ($P < 0.05$). The mean lengths of the postpartum period of the cows that treated with single injection of GnRH during the first, second or the third week postpartum and the control were 53.20 ± 1.10 , 40.00 ± 1.60 , 30.20 ± 1.1 and 75.20 ± 1.00 days, respectively (Fig. 2).

Fig. (3) explains that, the cows received a single injection of GnRH during the first, second or the third week postpartum had a similar number of services per conception (1.00 ± 0.00), which was significantly different ($P < 0.04$) from that of the control (1.40 ± 0.20). Furthermore, cows received single injection of GnRH during the first, second or third week postpartum had similar days open (Fig. 4), which were significantly different ($P < 0.001$)

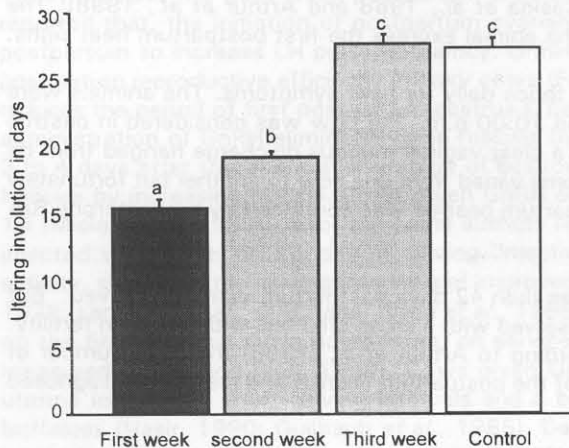


Fig. 1: The effect of injection of GnRH during the first, second or the third week postpartum on uterine involution (a, b, c $p < 0.005$).

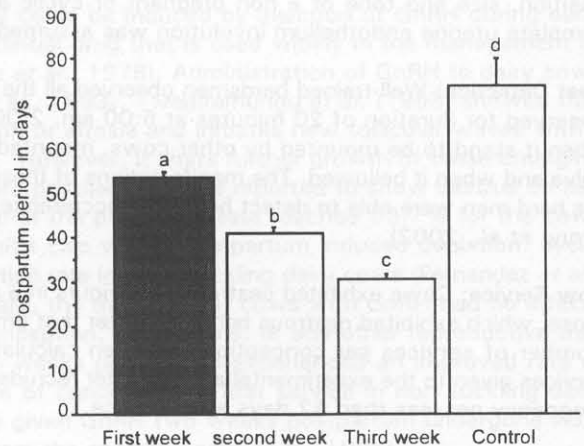


Fig. 2: The effect of injection of GnRH during the first, second or the third week postpartum on the length of postpartum period (a, b, c, d $p < 0.05$).

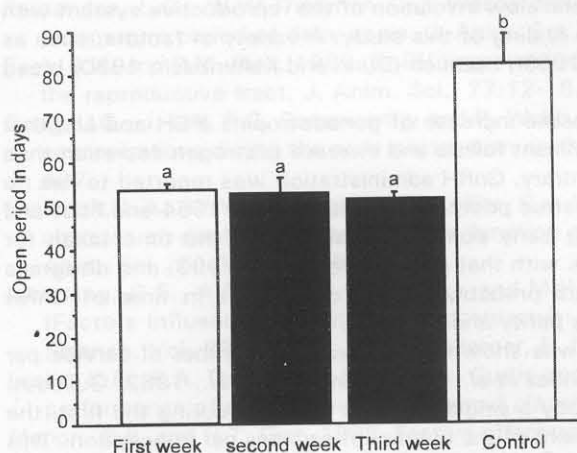


Fig.3: The effect of injection of GnRH during the first, second or the third week postpartum on days period (^{a,b} $p < 0.001$)

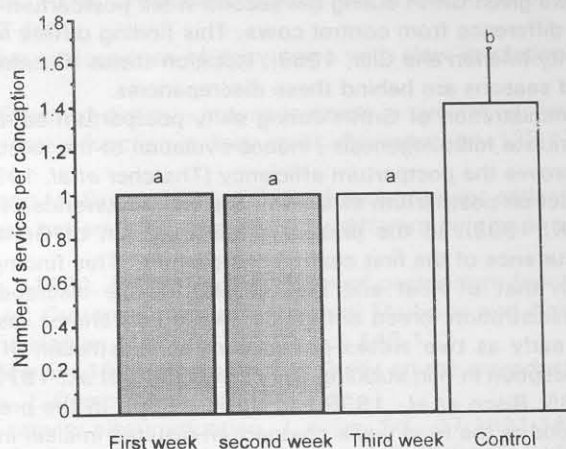


Fig. 4: The effect of injection of GnRH during the first, second or the third week postpartum on the number of services per conception (^{a,b} $p < 0.04$)

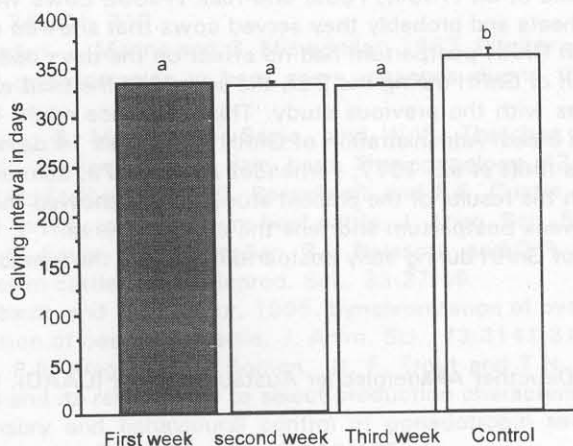


Fig. 5: The effect of injection of GnRH during the first, second or the third week postpartum on calving interval (^{a,b} $p < 0.001$).

from the control. The mean lengths of the days open of cows received a single injection of GnRH during the first, the second or the third week postpartum were 53.20 ± 1.10 , 52.30 ± 3.70 and 50.80 ± 1.20 days, respectively. Whereas, the days open of the controls were longer (81.40 ± 6.30 days).

Cows that received one injection of GnRH as early as the first, second or the third week postpartum showed a shorter calving interval as compared with the control ($P < 0.001$). The mean lengths of the calving interval of the cows that received one injection of GnRH during the first, second or the third week postpartum were 327.80 ± 1.30 , 326.00 ± 4.00 and 326.20 ± 1.40 days, respectively, which is shorter than that of the control (356.00 ± 6.10 days) Fig.5.

Discussion

The postpartum administration of GnRH reduces the interval from calving to complete uterine involution (Carvestany and Foote, 1985 and Abu-Ela *et al.*, 1986). Furthermore, cows given GnRH during the second week postpartum were reported to have an improved rate of uterine involution (Guilbault *et al.*, 1985 and Nasir, 1990). Administration of GnRH in dairy cows during the second week postpartum induced uterine involution (Britt *et al.*, 1977; Fernandez *et al.*, 1978; Lamming *et al.*, 1982; Thatcher *et al.* 1993 and Risco *et al.*, 1995). According to the results of the present study a single injection of GnRH during the first or second week postpartum enhanced uterine involution. This result matches with the previous studies. However, Leslie *et al.* (1984) showed that, dairy

cows given GnRH during the second week postpartum undergone slow involution of the reproductive system with no difference from control cows. This finding differs from the finding of this study. A variety of factors, such as parity (Marion and Gier, 1968), lactation status (Casida *et al.*, 1968), nutrition (Dunn and Kaltenbach, 1980) breed and seasons are behind these discrepancies.

Administration of GnRH during early postpartum causes an acute increase of gonadotropins (FSH and LH) that stimulate folliculogenesis, induce ovulation of the selected dominant follicle and increase oestrogen secretion thus improves the postpartum efficiency (Thatcher *et al.* 1993). Contrary, GnRH administration was reported to have no effect on postpartum efficiency and the occurrence of first oestrus postpartum (Leslie *et al.*, 1984 and Foote and Riek, 1999). In the present study injection of GnRH during early postpartum shortened the time taken for occurrence of the first oestrus postpartum. This finding agrees with that of Thatcher *et al.* (1993) and disagrees with that of Foote and Riek (1999). These discrepancies are probably due to differences in time of GnRH administration, breed difference, season difference, as well as parity and nutrition.

As early as two weeks postpartum administration of GnRH was shown to improve the number of services per conception in non suckling dairy cows (Britt *et al.*, 1977; Fernandez *et al.*, 1978; Lamming *et al.*, 1982; Guilbault, 1985; Risco *et al.*, 1995 and Nasir, 1999). In the present study a single injection of GnRH during the first, the second or the third week postpartum resulted in clear improvement of the number of services per conception. This result agrees with the results obtained from the above studies. This improvement is probably due to the release of a high quality DF. However, it was reported that the administration of GnRH to dairy cows postpartum had no effect on the number of services per conception (Etherington *et al.* 1985; Leslie *et al.*, 1984 and Foote and Riek, 1999), which is not consistent with the results of this study. The cows in the present study were not allowed to serve unless the cow exhibited the first postpartum heat at 42 days postpartum or more. However, in the reports of Etherington *et al.* (1985); Leslie *et al.* (1984); Foote and Riek (1999), cows were served immediately after expressing their first postpartum heats and probably they served cows that showed oestrus in less than 42 days. The treatment of dairy cows with GnRH postpartum had no effect on the days open (Foote and Riek, 1999). In the present study a single injection of GnRH during the first, the second or the third week postpartum reduced the days open. This finding disagrees with the previous study. This difference might be due to the time of GnRH injection, time of cow serving and breed. Administration of GnRH as early as 14 days postpartum shorten calving interval in non suckling dairy cows (Britt *et al.*, 1977; Fernandez *et al.*, 1978, Lamming *et al.*, 1982 and Risco *et al.*, 1995). This is consistent with the results of the present study, which showed that a single injection of GnRH during the first, second or third week postpartum shortens the calving interval.

It is concluded that, the injection of GnRH during early postpartum improve the reproductive performance of cross-bred dairy cows.

Acknowledgements

The authors are indebted to the Deuther Akademischer Austauschdienst (DAAD), for the grant supporting this study.

References

- Aboul-Ela, M.B. and F.E. El-Keraby, 1986. The effect of treatment with a GnRH analogue on postpartum reproductive performance in Friesian cows. *J. Anim. Reprod. Sci.*, 12:99-107.
- Arthur, G.H., D.E. Noaks, P. Harold and T.J. Parkison, 1998. *Vet. Reproduction and Obstetrics* (7th ed.), WB Saunders Co. Ltd.
- Bath, D.L., F.M. Dickinson, H.A. Tucker and R.D. Appleman, 1985. *Dairy cattle principles, practices, problems and profits*. (3rd ed.) Lea and Febiger, Philadelphia, 284-258.
- Britt, J.H., D.S. Harrison and D.A. Morrow, 1977. Frequency of ovarian cysts: reason for culling, and fertility in Holstein-Friesian cows given GnRH at two weeks after parturition. *Amer. J. Vet. Res.*, 38:749-751.
- Carvestany, D. and R.H. Foote, 1985. Reproductive performance of Holstein cows administered with GnRH analogue (Burserlin) 26 to 34 days postpartum. *J. Anim. Sci.*, 61:224-233.
- Casida, L.E., W.C. Graves, E.R. Hauser, J.W. Lauderdale, S. Saiduddin and W.J. Tyler, 1968. Studies on the postpartum cow. *Univ Wisconsin Res Bull.*, 270: 1.
- Dunn, T.G. and C.C. Kaltenbach, 1980. Nutrition and the postpartum interval of the ewe, sow and the cow. *J. Anim. Sci.*, 51 (suppl): 29-39.
- Elhag, M.A., 2003. Factors influencing postpartum reproductive traits in cross-bred dairy cows in the Sudan. M.Sc. Thesis, Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Khartoum.
- Etherington, W.G., S.W. Martin, I.R. Dohoo and W.T. Bosu, 1985. Interrelationships between postpartum events, hormonal therapy, reproductive abnormalities and reproduction performance in dairy cows. *Can. J. Comp. Med.*, 49: 261-267.
- FAO/IAEA, 1996. Progesterone radioimmunoassay protocol.

- Fernandez, L.C., W.W.Thatcher, C.J. Wilcox and E.P. Call, 1978. LH release in response to GnRH during the postpartum period of dairy cows. *J. Anim. Sci.*, 46:443-448.
- Foot, R.H. and P.M. Riek, 1999. GnRH improves reproductive performance of dairy cows with slow involution of the reproductive tract. *J. Anim. Sci.*, 77:12-16.
- Gong, J.G., J. Lee, P.C. Garnsworthy and R. Webb, 2002. Effect of dietary - induce increase in circulating insulin concentrations during the early postpartum on the reproductive function in dairy cows. *Reproduction*, 123:419-427.
- Guilbault, L.A., W.W. Thatcher, R.S. Collier, C.J. Wilcox and M. Drost, 1985. Effect of periparturient endocrine changes on postpartum reproductive function of Holstein heifers bred to genetically different service sire. *J. Anim. Sci.*, 61:1516-1526.
- Lamming, G.E., A.R. Peters, G.M. Riley and M.W. Fisher, 1982. Endocrine regulation of postpartum function (Factors influencing fertility in the postpartum cows). In: *Current Topics in Veterinary Medicine and Animal Science*. Vol. 20. Karg, H., Schallenberger, E. (eds). The Hague: Martinus Nijhoff, pp: 148-172.
- Leslie, K.E., P.A. Doig, W.T. Bosu, R.A. Curtis and S.W. Martin, 1984. The effect of GnRH on the reproductive performance of dairy cows with retained placenta. *Can. J. Comp. Med.*, 48:454-359.
- Marion, G.B. and H.T. Gier, 1968. Factors affecting bovine activity after parturition. *J. Anim. Sci.*, 27:1621-1626.
- Mialot, J.P., G. Laumonnier, C. Ponsart, H. Fauxpoint, E. Barassine, A.A. Ponter and F. Deletang, 1999. Postpartum subestrus in dairy cow: Comparison of treatment with PGF_{2α} or GnRH + PGF_{2α} + GnRH. *Theriogenology*, 52:901-911.
- Nasir, H.S.S., A.H. Willemse and D.F. Van de Wiel, 1990. Reproductive performance of Nili-Ravi buffaloes after single injection of GnRH. *J. Trop. Anim. Health Prod.*, 22:239-246.
- Nett, T.M., 1987. Function of the hybothalamic-hypophysial axis during the postpartum period ewes and cows. *J. Reprod. Fertil (Suppl.)*, 34:201- 213.
- Nett, T.M., D. Cermak, T. Braden, J. Manns and G. Miswender, 1987. Pituitary receptor for GnRH and estradiol, and pituitary content of gonadotropins in beef cows, changes during the estrous cycle. *Domis. Anim. Endocrinol.*, 4:123-132.
- Risco, C.A., R.L. De La Sota, G. Morns, J.D. Savio and W.W. Thatcher, 1995. Postpartum reproductive management of dairy cows in a large Florida dairy herd. *Theriogenology*, 43:1249-1258.
- Short, R.E., E.L. Bellow, R.B. Staihgmler, J.G. Berardinelli and E.E. Custer, 1990. Physiological mechanism controlling anestrous and fertility in postpartum beef cattle, *J. Anim. Sci.*, 55:799-816.
- Thatcher, W.W., M. Drost, J.D. Savio, K.L. Macmillan, R.L. Delasota and G.R. Morries, 1993. New clinical uses of GnRH and its analogues in cattle. *Anim. Reprod. Sci.*, 33:27-49.
- Twagiramungu, H., L.A. Guilbault and J.J. Dufour, 1995. Synchronization of ovarian follicular waves with a GnRH agonist to increase precrsion of oestrus in cattle. *J. Anim. Sci.*, 73:3141-3151.
- Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H. F. Trout and T.N. Lesch, 1982. A dairy cow body condition scoring system and its relationship to select production characteristics. *J. Dairy Sci*, 65: 495-499.
- Williams, G.L., 1990. Sensory and behavioural control of gonadotropin secretion during suckling-mediated anovulation in cows. *J. Reprod. Fertil. (Suppl.)* 49:463-475.
- Yavas, Y. and V. Walton, 2000. Postpartum cyclicity in suckled beef cows (review). *J. Theriogenology*, 54:25-55.