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CONCENTRATION OF PROGESTERONE, ESTRADIOL-17B, CALCIUM, PHOSPHORUS AND CA/P RATIO IN BLOOD SERUM OF FRIESIAN COWS DURING LATE PREGNANCY, PARTURITION AND POSTPARTUM PERIOD IN UPPER EGYPT

(With 2 Tables)

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مستوى هرموني البروجستيرون، ألأستراديول ١٧ بيتا ،الكالسيوم والفوسفور والنسبة بين الكالسيوم والفوسفور في سيرم دم ألأبقار الفريزيان خلال فترات الحمل، الولادة وما بعد الولادة في صعيد مصر

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اجريت هذه الدراسة بمحطة التجارب بقسم ألأنتاج الحيواني كلية الزراعة جامعة ألأزهرفرع اسيوط. اشتملت هذه الدراسة علىعدد ١٢ بقرة فريزيان وكان الهدف من هذه الدراسة متابعةً التغيرات في مستوي كلا من هرموني البروجستيرون، ألأستراديول١٧ بيتا ،الكالسيوم والفوسفور في سيرم الدم. أظهرت النتائج ارتفاع مستوي هرمون البروجستيرون قبل الولادة وكان أعلا مُستوي للهرمون في الأسبوع السابُّع بينما كان مستوي هرمون الاستراديول١٧ بيتا منخفض خلال هذه الفترة. اظهر البروجستيرون انخفاض معنوي عند الولادة في حين اظهر هرمون ألأستراديول١٧ بيتا زيادة معنوية في ألأسبوع الأخير قبل الولادة وعند الولادة. وعلى الجانب ألأخر اظهر كلا من البروجستيرون وألأستراديول ١٧ بيتا أنخفاض معنويا في الأسبوع الأول بعد الولادة ثما بدء هرمون البروجستيرون في الزيادة التدريجيه حتى وصل اعلا مستوى له في الأسبوع التاسع من الولادة بينما لم يكن هرمون الأستراديول ١٧ بيتا اتجاة واضح خلال هذه الفترة. أظهرت النتائج ارتفاع مستوى كلا من الكالسيوم والفسفورقبل الولادة وكان اعلامستوي لهما في ألأسبوع السابع، اظهرا كلا منهما انخفاض عند الولادة وكان هذا ألانخفاض معنويا بالنسبة الى الكالسيوم، أظهركلا من الكالسيوم والفوسفورزيادة تدريجيه من ألأسبوع ألأول بعد الولادة وكان اعلامستوي لهما في ألأسبوع التاسع بالنسبة للكالسيوم والسابع بالنسبة للفسفورفي الحيوانات المبكرة في الحمل اما بالنسبة للحيو أنات المتأخرة كأن اعلامستوى لها في ألأسبوع التاسع من الولادة. لم يكن هناك أختلاف واضح للنسبه بين الكالسيوم ألى الفوسفورقبل الولادة لكنها انخفضت انخفاض معنوى عندالولادة. انخفضت النسبة بين الكالسيوم الى الفوسفور انخفاض معنوى بالنسبة الى

الحيوانات المبكره فى الحمل خلال ألاسبوع ألأول من الميلاد' ثم ارتفعت ارتفاع تدريجي من الأسبوع الثالث بعد الولادة وكان اعلامستوي لهما فى ألاسبوع التاسع من الميلاد بينما لم يكن هناك اتجاة واضح خلال هذه الفترة للحيوانات المتأخرة فى الحمل.

SUMMARY

Twelve Friesian cows were used to study the serum changes of progesterone, estradiol-17B, calcium and phosphorus concentrations, in the periparturient. Serum progesterone level showed little changes among weeks 9,7 and 5 prepartum. Then the levels decreased significantly (P<0.05) among weeks 9, 7, 5 and 3 to reach a minimum level (P<0.01) at the timing parturition. The serum progesterone level kept minimum (< 1.0 ng/ml) up to weeks 5 and 9 after parturition in early and later conceived respectively. Then the level started to increase about 1 ng/ml 9 week after birth in early conceived. Serum estradiol17B increased progressively from 4.19±0.7pg/ml weeks 9 before birth to a beak (99.53 ± 25.98 pg/ml) on the day of parturition (P<0.05), the differences among weeks 9^{th} to 5^{th} prepartum were non significant Then the levels sharply drop to 2.83 ± 0.31 and 2.1 ± 0.27 pg/ml one week after birth (P<0.05) in early and later conceived respectively. The level remained low throughout the 9 weeks after birth in both early and later conceived. Calcium and phosphorus levels were highly (P<0.05) 7th prepartum, and then steadily decreased till parturition. In postpartum, there was general trend that the level of calcium increased gradually to reach maximum level at 9th week in both early and later conceived. While phosphorus level increased gradually to reach maximum at 7th and 9th weeks in early and later conceived respectively. changes in Ca/p ratio in the cow was not significantly from 9th to 3rd weeks prepartum. While the Ca/p ratio dropped drastically (P<0.01) at parturition. Postpartum Ca/p ratio increased gradually advances reached maximum ratio at 9th week postpartum in early-conceived group. On the other hand, the Ca/P ratio in late conceived group reached maximum at 7th week postpartum.

Key words: Cows, periparturient, progesterone, Estradiol-17β, Calcium, Phosphorus.

INTRODUCTION

Factors, which initiate birth and thereby terminate pregnancy after a constant length of gestation for a givern species are still imperfectly undeterred. Progesterone is the predominant hormone

secreted by the corpus luteum during the estrous cycle and pregnancy, (Reimers, et al 1980, Tomas and Dobson, 1989, Roy, et al, 1990, and Dijkhuizen and VanErdenburg, 1997). Serum progesterone remained low from parturition until 2-3 days after first ovulation, when it increased again. Contrarly, plasma estradiol -17β concentration was high few days before parturition, reached a peak at the time of birth (Horst et al 1978, Hollis et al., 1981and Crowe, et al , (2001). During 1st week postpartum, serum estradiol concentration was lower (Stevensone and Britt, 1979 Prakash and Madon, 1986, Badr et al., 2001). The fall in both progesterone and estrogen levels after calving results in the removal of the hypothalamic pituitary block and retains it resposiveness by approximately the 10th days postpartum (Lamming, et al., 1982, Drawsh, et al, 2001 and Crowe, et al , 2001).

The mean time from parturition to the first the postpartum estrous ranged from 52 to 68 day (Perkins and Kidder, 1963, Bastedis, et al., 1984, Ali, 1992 Crow, et al, 2001 and Badr et al., 2001). Schams et al (1978) found that 50% of cows showed short cycles during the early postpartum period. It has been suggested that the lack of progesterone concentration (Schirar and Martinet, 1982.) or the immature corpus luteum (Morrow et al, 1969, Roy, et al, 1990 and Crowe, et al, 2001) during the early postpartum period may be the cause of the short cycles. The duration of postpartum anoestrous can be affected by many factors such as nutrition, age, lactation and suckling (Lishman, et al; 1979; Laster, et al 1973 and Edgerton, 1980).

Calcium and phosphorus metabolism is essential for mineralization of fetal skeleton during preneonatal period. They concerned also with numerous metabolic processes in connection with carbohydrate, skeletal muscle and fat metabolism and also involved in many co-enzum, (Tainturer et al, 1984 and Horst, 1986.

The following study was designed to determine the sequential serum levels of progesterone, estradiol 17β , Calcium and phosphors concentration during the late pregnancy, parturition and during postpartum period in Friesian cows.

MATERIAL and METHODS

Twelve Friesian cows, aged 3-6 years with body weight of 325 - 400kg were used in the present study. The animals were kept in the experimental Station of Animal Production Department of Agriculture, Alazhar Unvirsity, Assiut Branch, Egypt. The animals were fed according their body weight and the physiological state. All animals

were fed on Egyptian clover (Trifolium alxandrinum) from December to May, while in summer season they were fed on grean forage (Daraw). In addition, wheat straw and concentrated mixture were added. The concentrated mixture consisted of yellow corn, wheat bran, molasses, cottn seed meal, sun flower, stone and salt. The animals were allowed to drink fresh water along the day. All animals were kept in open free-stalls, excepted place of shed reed with thatch to protect animals from solardation. Two months after birth, a fertile bull was introduced to cows for natural mating. Two months after later, all the animals were examinated Ultrasonographically for pregnancy diagensis. According to the result of pregnancy diagnosis, cows were classified into: 1- Animals conceived early (< 90 days postpartum). 2- Animals conceived later (> 90 days postpartum).

Blood samples were collected from the jugular vein using 10ml glass tubes at 9, 7, 5 and 3 weeks prepartum, at day of parturition, and at 1, 3, 5, 7 and 9 weeks postpartum. Samples were centrifuged at 4000 rpm for 15 min and stored at -20 °c until analysed for calcium, phosphorus, progesterone and estradiol17β. Progesterone and estradiol17β concentrations were estimated by direct radioimmunoassay (RIA) using Coat A-count kits Calcium and phosphorus level were estimated biochemically using commercial Kits provided by Stanbio as described by Lehman *et al* (1984).

Statistical analysis were carried out according to SAS (1988) users Guide, tested using the Duncan,s Mltiple Range Test as described, Walpole, (1974).

RESULTS

The changes in the progesterone concentrations of the Friesian cows during prepartum, parturition and postpartum periods are presented in table (1). The serum progesterone level showed little changes between Week 9 and Week 7 prepartum, then the levels decreased significantly (P<0.01) among weeks 7, 5 and 3 to reach a minimum level (P<0.01) at the time of parturition. The serum progesterone level kept minimum (< 1.0 ng/ml) up to weeks 5 in early and later conceived. Then the level started to increase above 1 ng/ml 9 week after birth in early conceived cows.

The serum estradiol17 β increased progressively from 4.19 \pm 0.7pg/ml weeks 9 before birth to a beak (99.53 \pm 25.98pg/ml) on the day of parturition (P<0.01). Then the levels sharply dropped to 2.83 \pm 0.31

and $2.1\pm~0.27$ pg/ml one week after birth (P<0.01) in early and later conceived, respectively. The level remained low throughout the 9 weeks after birth in both early and later conceived. The differences among weeks 1st to 7th postpartum were not significant, also the difference between early and later conceived was non significant during postpartum.

Serum concentration of calcium during prepartum, parturition and postpartum are illustrated in (Table 2). Calcium level was high 7 Weeks prepartum, and then steadily decreased till parturition (P<0.05). In the postpartum period, there was a general trend that the level of calcium increased gradually to reach maximum level 9 weeks postpartum in both early and later conceived cows.

Serum concentration of phoshorus during prepartum, parturition and postpartum, are inllustrated in (Table 2). The Phosphorus level showed a little changes before parturition. It decreased significantly after birth (p<0.05). The drope in concentration was more pronunced in late conceived cows. Difference between early and late conceived was significant at week 7 (p<0.05).

Tables 2, showed Ca/P ratio during prepartum, parturition and postpartum in the both early and late conceived cows. The ratio decreased gradually from week 9 befor birth to reach a minimum at parurition (p<0.05), then the ratio increased gradually to reach a maximum 7 and 9 weeks after birth in late and early conceived cows (p<0.05).

DISUCSSION

The periparturient changes in progesterone concentration is in agreement with the results by Stevenson and Britt, (1979) and Badr et al, (2001). They found that the serum progesterone remained less than 0.2 ng/ml from parturition until 2-3 days after first ovulation, when it increased to greater than 1 ng/ml. Decrease in progesterone level presumably removing the blocking mechanism on the myometrium. The stimulation for this change is dependent initially upon a sharp rise in the secretion of cortisol by the fetal adrenals to the release of fetal ACTH. The reason for the sudden release of ACTH is not full understood. It has been postulated that it reflects the response of the fetal hypothalamus. The stimulus may be related to the fetus out-growing the ability of the placenta to supply adequate nutrients or provide adequate gaseous exchange, Arthur, et al, (1989). In the present study, the level of

progesterone strated to increased 7th week after birth in early-conceived indicating the begin of ovarian activity. Other studies indicated that the postpartum ovarian activity beginned between days50 and 90 postparum (Zin,tzen, 1972, Mther, *et al* 1978 and Ali, 1992)

The periparturient changes in estradiol -17 β concentration is in agreement with the results of (Agthe and Kolm, 1975, Hollis, et al, 1981, Prakash and Madan, 1986, Abdo, et al, 1991, Eissa, et al 1995 and Badr, et al, 2001). The rise in fetal cortisol stimulates the conversion of placentally drived progesterone to estradiol 17 β by activating the placental enzyme 17 α -hydroxylase; this hydroxylates progesterone via androstenedione to estradiol 17 β . The consequences of the rise in estadio 17 β and decline progesterone in the peripheral circulation are threefold. First, estradiol have a direct effect upon the myometrium, increasing its responsiveness to oxytocin, second, they produce softening of the cervix by altering the structure of collagen fibres, third, they act upon the cotledon-caruncle complex to stimulate the production and release of PG2 α , (Liggins, et al; 1977). The latter change is inuced by the activation of the enzyme phospholipase A2 stimulates by the decline in progesterone and rise estradiol 17 β .

The periparturient changes in Calcium concentration is in agreement with the results by Nordin, (1976), Ferrel, et al. (1982), Ali (1992), and Badr (2001). They found a fall in calcium level in late pregnancy, which was attributed to increase of estrogens and adrenal corticoid hormones causing hypocalcaemic effect during the late gestation period this was confrmed by Ali (1992) reported that cows conceived after day 90 days postpartum had higher calcium serum level than those conceived before that time. Osman, (1985) found that serum calcium level was significantly higher in cyclic postpartum cows than those with inactive ovaries. Nordin, (1976) found that the decrease calcium concentration in plasma may be also due to reduction feed intake in late pregnancy or the high endogenous estrogen secretion. The homeostatic or physiological mechanisms regulating serum calcium concentration are more effective then those for phosphorus or most other minerals. In most species, serum calcium is controlled by the action of parathyroid hormone calcitonin and the active metabolite of vitamin D (McDowell, 1985, Horst, 1986 and Ciaramella, et al 2000).

The periparturient changes in phosphorus concentration is in agreement with the results by Samad et al, (1980), Zintzen (1972), and Ali (1992). Medway et al (1969) mentioned that, phosphorus is one of the feed stuff, which appear to influence the appetite and so phosphorus

deficiency under maintenance requirement has a great influence on food consumption and consequentty on the fertility

The differences among weeks in Ca/P may be attributed to the differences in calcium and phosphorus concentration. These results are in agreement with the stement of Ali (1992), who stated that this Ca/P ratio reflects the absolute levels of calcium and phosphorus in the serum cows. He lso added, that the serum Ca/P ratio were wider in groups of cows that should latent ovarian activity than the early group. Olson et al (1986) found that absolute levels of calcium and phosphorus are more important for reproduction than the Ca/P ratio. Gerloff and Morrow, (1986) suggested that absolute amount of Ca, p and vit D in the diet were more important than Ca/P ratio.

Table 1: Changes in serum concentrations of Progesterone and Estradiol 17ß in Friasien cows during late pregnancy, parturtition and postpartum period in Friesian cows.

Weeks	Progester	one ng/ml	Estradiol 178 pg/ml			
Prepartum						
-9	3.83 =	± 0.22ª	4.19 ±0.57 ^b			
-7	4.56 =	± 0.21 ^a	9.16± 0.87 ^b			
-5	3.22 ± 0.23^{a}		12.18 ±1.7°			
-3	2.2 ± 0.18 b		97.86 ±8.6 a			
Parturition	0.072 ± 0.00^{d}		99.53± 25.98 a			
Postpartum	Early conceived	L.ater conceived	Early conceived	Later conceived		
1	0.045±0.01d	0.041 ±0.01 ^d	2.83± 0.45 ^b	2.31±0.27 ^b		
3	0.31± 0.01 ^{cd}	0.27±0.032d ^{cd}	6.55 ±0.59 b	4.97±0.65 b		
5	0.72± 0.16 °	0.66±0.06 ed	3.1± 0.26 b	3.14±0.14 b		
7	1.08± 0.16 °	0.75±0.1 ^{cd}	3.58 ±0.96 b	2.1±0.36 b		
9	1.2 ± 0.14^{e}	0.83± 0.19 ^{cd}	3.26 ±1.6 b	0.98±0.21 b		

a, b, c, d,: Values in the same rows with different superscripte are different (P<0.01).

Table 2: Changes in serum concentrations of Calcium, Phosphorus and Ca/P ratio in Friasien cows during late pregnancy, parturtitieriod and postpartum period in Friesian cows.

Weeks	Calcium mg/ml		Phosphorus mg/ml		Cal/P ratio	
Prepartum			į			
-9	6.5 ±0.51 a		4.95±0.51 a		1.13 ±0.15 a	
-7	7.1 ±0.41 a		5.71±0.49 a		1.2± 0.16 a	
-5	5.5±1.1 ^{ab}		4.74±0.39 a		1.2± 0.1 a	
-3	5.0±0.9 ^{ab}		4.95±0.38 a		1.1± 0.15 a	
Parturition	3.63±0.4 b		4.68±0.62 a		0.78±0.18 b	
Postpartum	Early Conceived	Later Conceived	Early conceived	Later conceived	Early conceived.	Later conceived
1	3.92±0.36 b	3.4±0.44 b	3.2±1.1ab	2.9±0.3 ^b	0.71±0.2 b	1.16±0.16 ^b
3	4.13 ±0.56ab	3.63 ±03 ^b	3.3±0.3ab	2.5±0.2 ^b	0.99±0.2 b	1.14±0.23 ^b
5	4.25 ±0.63ab	3.73 ±0.2 ^b	3.2±0.2ab	2.9±0.3 ^b	1.42±01 ^a	1.3±0.1ab
7	5.42 ±0.23 a	4.7 ± 0.33^{ab}	5.2±0.32 a	3.0±0.16 ^b	1.32 ±0.13 ab	1.6± 0.1 a
9	6.62 ±0.4 a	5.6 ±0.46 ab	4.1±0.5 a	4.4±0.1 a	1.62 ±0.16 a	1.3±0.29 a

a & b: Values in the same rows with different superscripte are different (P<0.05).

REFERENCES

- Abdo, G.A.; Njuguna, O.M.; Fredriksson, G. and A. Madej, (1991): Level of oestrone sulphate during pregnancy in different breeds of cows and its possible association with retained foetal membranes. Acta. Vet Scand. 32: 183-188.
- Agthe, O. and H.P. Kolm, (1975): Oestrogen and progesterone levels in the blood plasma of cows with normal parturition or with a retained placenta. J. Reprod. Fert. 43: 163-166.
- Ali, A.M., (1992): Some studies on the postpartum period in cattle.M.Sc. Thesis, Fac. Of Vet. Med. Assiut, Univ. Egypt.
- Arthur, G.H.; Noakes, D.E. and Pearson, H. (1989): Veterinary Reproduction and Obstetrics.Parturition and the care of Parturient Animals. PP. 132-160, 6th Ed. Bailliere Tindall, Great Britain.
- Badr, H.M., Ashour, A.M. and Solouma, G. M. (2001): Serum Concentrations of Progesterone and Esradio-17β During Late Gestation, Parturition and postpartum and Its Relation to Ovarian Activits in Egyptian Buffaloes. Al-Azhar J. Agric. Res. Vol. 33: PP. 27-44.

- Bastidas, P.J., Troconiz, O. Silva (1984): Effect of restricted suckling and ovarian activity and uterine involution in Brahman cows. Theriogenology 21: 525-531.
- Ciaramella, P.; Piantedosi, D.; De Luna,; Oliva, G.; Consalvo, F. and A. Persechino. (2000): Biochemical indicators of bone metabolic activity in buffalo (Bubalus Bubalis) during late pregnancy and early lactation. J. Vet Med. Phosiol. Pathol. Clin. Med.
- Crowe, M.A.; Enright, W.J.; Boland M.P. and, J.F. Roche (2001).: Follicular growth and serum follicle- stimulating hormone (FSH) responses to recombinant bovine FSH in GnRH-immunized anoestrus heifers. Anim. Sci. 73: 115-122.
- Darwash, A.O.; Lamming, G.E. and L. M., Hicking (2001): Oestrus in relation to peak oestradiol levels in ovariectomized Galloway cows. Anim. Sci. 72: 401-405.
- Dijkhuizen, T.J. and vanErdenburg, F.C.M. (1997): Behaviural signs of estrus during pregnancy and lactating dairy cows. Veterinary Quartrly 19: 194-196.
- Edgerton, L.A. (1980): Effect of lactation upon the postpartum interval. J. Anim. Sci. 51: 40-52.
- Eissa, H.M., El-Belely, M.S., Ghoneim, I.M. and O.H. Ezzo (1995):
 Plasmaprogesterone, oestrodiol 17beta, oestrone sulphate, corticosteroids and a metabolite of PGF₂ alpha: evolution throughout pregnancy, before, during andafter parturation buffalo cows. Vet. Res. 26 (4): 310-318.
- Ferrel, G.E., B.B. Laster and R.L. Prior (1982): Mineral accretion during prenatal growth of cattle. J. Anim. Sci.; 54: 618-624.
- Gerloff, B. J. and Morrow, D.A. (1986): Effect of nutrition on reproduction in dairy cows. Current therapy in Theriogenology PP. 310-320. Edited by Morrow, D.A.; 2nd Ed. W.B. Saunders Company, Philadelphia.
- Hollis, B.W.; Draper, H.H.; J.H. and J.H., Burton. (1981): A hormonal assessment of bovine parturient paresis: evidence for a role of oestrogen. J. Endo. Crinol. 88: 161-171.
- Horst, R.L. (1986): Regulation of calcium and phosphorus homeostasis in dairy cow. J. Dairy Sci. 69: 604.
- Horst, R.L.; Jorgensen N.A. and H. F. Deluca H. F. (1978): Plasma 1.25-dihydroxy. Vitamin D and parathyroid hormone levels in paretic dairy cows. Am. J. Physiol. 235: 634-637.

- Lamming, G., A.R. Peters, G.M. Riley and M. W. Fisher (1982): Factors influencing fertility in the postpartum cow. Eds. Karg, H and E. Schallenberger, PP. 173-186.
- Laster, D.B., H.A. Climp, K.E. Gergory, (1973): Effects of early weaning on postpartum reproduction of cows. J.Anim. Sci. 36: 734-740.
- Lehman, H.P. and J. B. Henry, (1984): In clinical diagnosis and mangement by Laboratory Methods, 17 thed.; J.B. Henry, Ed. Saunders, Philadelphia pp. 1431-1438.
- Liggins, G.C; Fairclough, R.J., Grieves, S.A., Forster, C.S. and Knox, B.S. (1977): In The fetus and Birth Ciba Foundation Symposium 47, eds J. Knight and M.O, Connor, P.S. Amsterdam: Elsevier North Holland.
- Lishman, A.W.; Allison, S.M.; Fofewell, R.L.; Butcher, R.L. and E.K. Inskeep,. (1979): Follicular development and function of induced corpora lutea in underfed cows, J. Anim. Sci. 48: 867-875.
- Mather, E.C.; Camper, P.M.; Vabdat, F.; Whitmores, H.L. and Gustaffson, B.G. (1978): Assessment of ovarian activity in the postpartum dairy cow by use of a milk progesterone assay. Theiogenology., 10: 119.
- McDowell, I.R. (1985): Nutrition Grazing Ruminants in Warm Climmates Academic Press New York. 2nd Ed.
- Medway, W.; Prier, J.E. and Wilkinson, J. S. (1969): A textbook of veterinary clinical pathology. Pp. 14-60, Williams and Wilkins Co., Boltimore USA.
- Morrow, D.A.; Roberts, S.J. and McEntee, K. (1969): A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. Cornell, Vet., 59: 135.
- Nordin, B.E.C. (1976): Calcium, phosphorus and magnesium metabolsm, clinical physiology and diagnostic procedures. Churchill Livingtsone, Edinburgh, London. 1st Ed. Morrow, D.A. 2nd Ed., W.B. Saunders company, philadephia.
- Olson, J.D.; Bretzlaff, K.N.; Mortimer, R.G. and Ball, L. (1986): The metritis-pyometra complex. Current Therapy in Theriogenology. PP (227-250). Ed. Morrow, D.A.2nd Ed., W.B. Saunders company, philadephia.
- Osman, A.M.; El-Naggar; M.A.; Farrag, A.A. and Shehata, S.H. (1985):
 Ovarian Inactivity among Egyptian cows and buffaloes. Assiut
 Vet. Med. J., 14: 219.

- Perkins, J.L. and H.S. Kidder. (1963): Relation of uterine involution and postpartum interval to reproductive efficincy in beef cattle. J.Anim. Sci. 22: 313-315.
- Prakash, B.S. and Madon, M.L. (1986): Peripheral plasma oestrogen-17_B progesterone and cortisol in buffaloes induced to calve with Dexamethasone vetoestrol. Anim. Reprod. Sci. 11: 111-122.
- Remimers, T.J.; Smith, R.D. and Foote, R.H. (1980): Milk progesterone testing to detection reproductive status of dairy cows. XI Inter. Congr., on disease of cattle. Tel-Aviv. 906.
- Roy, G.P., Singh, A.P., Akhtar, M.H., Prasad, K.M., Singh, R.B. and Sinha, S.N. (1990): Incidence of mid-cycle estrus in cattle and buffaloes. Indian J. of Anim. Reprod. 11-158
- Samad, A.; Ali, K.M. and Rahman, A. (1980): Studies on certain blood constituents of anoestrus cattle Ind. Vet. J. 57:135.
- SAS. (1988): SAS Users Guide: Statistics. SAS Inst., Inc., Cary, NC.
- Schams, D.; Schallenberger, E.; Menzer, C.; Stangl, J. Zottmeir, K., Hoffmann, B. and Karg, H. (1978): Profile of L.H, F.S.H. and progesterone in postpartum dairy cows and their relationship to the commencement of cyclic function. Theriogenology, 10: 453.
- Schirar, A, and L. Martinez, (1982): In, factors influencing fertility in the postpartum cow. Eds, Karg, H. and H. and E. Schattenberger, pp. 67-94
- Stevenson, J.S. and Britt, J.D. (1979): Relationship among luteinizing hormone, estradiol, progesterone, glucocorticod milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci., 48: 570.
- Tainturier, D.; Bravn, J.P.; Rico, A.G. and Thouvenot, J. P. (1984): Varian in blood composition in dairy cows during pregnancy and after calving. Vet. Sci.; 37: 129-131.
- Thomas, I. And Dobson, H. (1989): Oestrus during pregnancy in the cow. Veterinary Record. 124: 387.
- Walpole, R.E. (1974): Introduction Statistics. 2nd Ed. Macmillar Publishing Co. Inc. New York, Collier Macmillan Pub. London PP. 277-278.
- Zintzen, H. (1972): Fertility and Nutrtion in dairy cows. Lecture given at the 11th congress of the Souh African Society of Animal production, Johannesberg (Cited by Shehata. 1983).