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**COMPARATIVE EFFICACY OF DIFFERENT
INTRAUTERINE THERAPIES ON REPRODUCTIVE
EFFECIENCY IN DAIRY COWS AFFECTED
WITH ENDOMETRITIS**

(With 3 Tables)

By

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**مقارنة فاعلية مختلف العلاجات داخل الرحم علي الكفاءة التناسلية
التي تعاني من التهابات بطانة الرحم**

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أجريت هذه الدراسة بهدف مقارنة فاعلية مختلف العلاجات داخل الرحم على الكفاءة التناسلية للأبقار التي تعاني من التهابات بطانة الرحم. تمت هذه الدراسة على ٩٠ بقرة حلب من نوع هولستين فريزان (أعمارها تتراوح بين ٤-٧ سنوات) وكانت في الفترة من ٨٠ إلى ١٠٠ يوم بعد الولادة. تم تقسيم هذه الأبقار إلى خمس مجموعات متساوية في العدد (١٨ بقرة لكل مجموعة). كانت المجموعة الأولى ضابطة والتي تم علاجها بـ ١٠٠ مللي من محلول ملح فسيولوجي داخل الرحم. والمجموعة الثانية تم علاجها بـ ٥٠ مللي من بلازما دم الحيوان نفسه. والمجموعة الثالثة تم علاجها بـ ١٠٠ مللي من مصلى اللبأ. تم تكرار العلاج في هذه المجموعات الثلاث كل ٤٨ ساعة لثلاث مرات. والمجموعة الرابعة والخامسة تم علاجها بـ ١٥٠٠ ملجم من أكسي تتراسيكلين (متريجيت) و ١٠٠ مللي من محلول اللوتاجين على التوالي كجرعة واحدة في حالة استمرار الإلتهاب الرحمي. تم تكرار العلاج نفسه لثلاث مرات على الأقل على الحيوان نفسه. أظهرت نتائج هذه الدراسة أن نسبة نجاح العلاج بعد المرة الأولى ٤١,١% وأن نسبة النجاح الكلية (بعد ثلاث مرات علاج) بلغت ٦١,١%. كذلك وجد أن كل من العلاج وحالة الولادة لهما تأثير معنوي على الفترة ما بين الولادة وحدوث الحمل. كذلك لوحظ أن أعلى نسبة نجاح بعد أول علاج وبعد ثلاث مرات وأن أقصر فترة ما بين الولادة وحدوث الحمل كانت لكل من المجموعتين اللتان تم علاجهما بالمتريجيت ومصلى اللبأ. بينما كانت الفترة بين الولادة وحدوث الحمل أطول معنويًا بالمجموعة المعالجة باللوتاجين مقارنة بالمجموعة المعالجة بالمتريجيت. كما وجد أن حالة الولادة كان لها تأثير معنوي على فترة ما بين الولادة وحدوث الحمل فكانت أطول معنويًا في الحيوانات التي تم مساعدتها بالولادة (١٩١,٣٣ ± ٥,١٥) عن الحيوانات التي ولدت طبيعيًا (١٧٥,٤٧ ± ٥,١٩). ويتضح من هذه النتائج أن علاج الإلتهابات الرحمية باستخدام أي من المتريجيت أو مصلى اللبأ أدى إلى تحسين نسبة حدوث الحمل و تقصير الفترة ما بين الولادة وحدوث الحمل

في الأبقار الحلوب، وعند المقارنة بين هاتين المجموعتين كان الفرق غير معنوياً ولكن من ناحية الخسارة الاقتصادية نتيجة ثمن العلاج وكذلك إعدام اللبن غير الصالح للاستهلاك البشري فإنه يفضل استخدام مصل اللبأ في علاج التهابات الرحمية الخفيفة في الأبقار الحلوب.

SUMMARY

A study was conducted to compare the effectiveness of various intrauterine therapies on enhancing the conception rate of dairy cows affected with endometritis. Ninety Holstein-Friesian cows (4-7 years of age) at about 80 to 120 days postpartum were included in this study and randomly allocated into five equal treatment groups (n = 18). An intrauterine infusion of 100 ml normal saline (control); 50 ml homologous plasma or 100 ml colostric serum every 48 hours for three times; OTC infused intrauterine as a single dose; and 100 ml, of Lotagen solution infused intrauterine as a single treatment dose. If the cervico-vaginal mucus condition was not clear, the cow was treated again for a maximum of three times by the same treatment. The successfully treated cows were inseminated. The overall success rate of treatments was 61.1% and the success rate after 1st treatment was 41.1%. There was a high significant effect ($p < 0.01$) of treatment and calving condition on interval from calving to conception. The higher success rate after 1st treatment (61.1% and 50.0%); overall success rate (83.3 % and 72.2 %) and shorter interval from calving to conception (157.72 ± 8.57 and 171.00 ± 9.58 days) were observed in OTC and colostric serum treated groups, respectively. However, the interval from calving to conception for Lotagen treated group was significantly longer than oxytetracycline group ($p < 0.05$) and shorter than control and plasma treated groups. Calving condition had a significant effect ($p < 0.05$), especially on the interval from calving to conception. The interval was longer for cows with history of abnormal calving or abnormal puerperium (191.33 ± 5.15 vs. 175.47 ± 5.19 days). These results indicate that intrauterine infusion with either OTC or colostric serum was effective to improve the pregnancy rate and to shorten the interval between calving and conception in dairy cows affected with mild endometritis. Economic losses due to cost and milk disposals may argue against the use of OTC and for the use of colostric serum in cases of mild endometritis.

Key words: Endometritis, Colostric serum, plasma, Oxytetracycline, Lotagen.

INTRODUCTION

Endometritis in breeding cattle occurs during the postpartum period and is associated with contamination of the reproductive tract as a result of breaking the physical and functional barriers (BonDurant, 1999; Dhaliwal *et al.*, 2001). The uterine defense mechanisms (UDMs) against contaminating microorganisms are maintained in several ways: anatomically by simple or pseudo-stratified columnar epithelium covering the endometrium; chemically by mucus secretions from the endometrial glands; immunologically, through the actions of polymorphonuclear inflammatory cells (PMNs) and humeral antibodies (Watson *et al.*, 1990; Butt *et al.*, 1991; Dhaliwal *et al.*, 2001). Disruption of these mechanisms allows opportunistic pathogens to colonize the endometrium and cause endometritis (Hussain *et al.*, 1990). Most healthy cows are able to clear the uterus of bacterial contamination in the first 2 to 3 weeks after calving (Hartigan *et al.*, 1974). However, in those cows unable to eliminate bacterial contamination, endometritis may develop subsequently.

Endometritis is deleterious for reproductive performance of dairy cows. The clinical consequences of endometritis include a reduction in fertility as measured by calving to conception intervals and first service conception rates (Bonnett and Martin, 1995; Knutti *et al.*, 2000). Optimal uterine environment is critically important for the viability of fertile spermatozoa and development of embryo (Srecnan and Behan, 1974).

A generally conventional series of cellular and chemical responses constitute the inflammatory reaction. In addition, for the surface area that is exposed to environmental flora at the mucosal surfaces, an effective system of mucosal defense is developed (BonDurant 1999). The uterus is a part of common mucosal immune systems. It contains the full range of lympho-haemopoietic cells and molecular regulators required to generate and elicit adaptive (antigen-specific) immunity (Wira *et al.*, 1999; Robertson, 2000). In addition, the synthesis of Immunoglobulin, classes IgA, IgG and IgM reflects both the extent of the endometrial inflammatory process in the face of microbial challenge and its chances of clinical recovery (Dhaliwal *et al.*, 1998). The magnitude or quality of uterine response to exogenous antigen is not

only influenced by nature of antigen, route of sensitization and the use of adjuvant (Parr and Parr, 1999; Dhaliwal *et al.*, 2001) but ovarian hormones and endocrine status have considerable effects (Wira *et al.*, 1999).

The uterine immune axis holds the key to solving major problems in female reproductive health including infertility and sexually transmitted diseases (Robertson, 2000). The immune response in the uterus may be adversely affected by several therapeutic strategies commonly used to treat endometritis in bovine species (Hussain and Daniel, 1991). Intrauterine administration of antibiotics, antiseptics and disinfectants suppresses uterine leukocytes activity (Gustafson, 1984; Nakao *et al.*, 1988). Conversely, several intrauterine chemotherapeutic agents may stimulate the uterine defense mechanism (e.g. lugol's iodine and polyvinyl pyrrolidanc). However, their use should be discouraged since they cause endometrial fibrosis (Nakao *et al.*, 1988). The failure of most currently used treatments to eliminate bacterial infection from uterus should direct research to the evaluation of alternative therapies for treatment of uterine infection. Recently alternative therapies that can stimulate the natural uterine defense mechanism have been suggested. These include immune-modulators, serum, plasma or hyperactive immune serum (Subandrio and Noakes, 1997; Singh *et al.*, 2000 and Dhaliwal *et al.*, 2001). The present study was designed to compare the effectiveness of various intrauterine therapies on enhancing conception rate of dairy cows affected with endometritis.

MATERIALS and METHODS

Ninety Holstein-Friesian cows (4-7 years of age) at about 80 to 120 days postpartum, kept under field conditions were included in this study. A thorough examination of the reproductive system of each animal by vaginal and trans-rectal palpation was performed. The selected animals were apparently healthy normally cycling cows and the cervico-vaginal mucus was abnormal at time of estrus. The mucus was cloudy and contained very mild to moderate white flakes indicating the cows were suffering from mild endometritis as described by Murray *et al.* (1990). Each cow's identity, parity, calving date, course of calving and puerperal period, and date of gynecological examination and clinical findings were recorded. The cows were randomly allocated to one of five

treatment groups. In each group, the cows (n = 18) on day of estrus were given one of the following treatments. An intrauterine infusion of 100 ml normal saline (control, group A); 50 ml homologous plasma (group B) or 100 ml colostric serum (group C) every 48 hours for three times; Oxytetracycline hydrochloride (OTC, 1500 mg, Metrijet ®. Intervet. UK) infused intrauterine as a single dose (group D); and condensed metacresolsulphonic acid and formaldehyde (100 ml of 4% Lotagen ® solution, Schering-plough Animal Health) infused intrauterine as a single treatment dose (group E). Each cow was re-examined again on subsequent estrus and the condition of cervico-vaginal mucus was assessed. If the cervico-vaginal mucus condition was not clear, the cow was treated again for a maximum of three times by the same treatment and a record was made indicating that this was the second or third treatment as appropriate. Once the cow was treated successfully (with clear cervico-vaginal mucus), it was inseminated. If the animal failed to conceive and returned to estrus, the insemination was repeated for a maximum of two subsequent estrus. Animals that did not respond to treatment were not inseminated. Pregnancy diagnosis conducted at 45 to 60 days after breeding. The following details were recorded, success rate after each treatment, date of first service after treatment, overall pregnancy rate (up to 210 days postpartum) and interval from calving to conception. Animals that did not respond to treatment or failed to conceive after three cycles were assigned a 210 days as days open.

The effect of different treatments and calving condition on the interval from calving to conception was analyzed statistically using analysis of variance (ANOVA). Duncan's New Multiple Range Test was applied to compare the differences between the means. The success rates and pregnancy rates were analyzed using Chi-square.

RESULTS

The overall means and standard errors (S.E) for some reproductive traits of tested cows in different treatment groups are shown in Table (1). There was no significant differences between treatment groups for the number of previous calvings; postpartum days at treatment and percentage of abnormal calving in each treatment group.

Analysis of variance (Tables 3) showed that the type of treatment and calving condition had a significant effect ($P < 0.01$) on interval from calving to conception. The overall success rate after the first treatment

was 41.1% and this increased to 61.1% after the third treatment. The higher values of success rate after 1st treatment (61.1% and 50.0 %); overall success rate (83.3 % and 72.2%); pregnancy rate (72.2 % and 55.5%) and shorter intervals from calving to conception (157.72 ± 8.57 and 171.00 ± 9.58 days) were observed in OTC and colostric serum treated groups (Group D & C), respectively (Table 2). The comparison between overall success rates and pregnancy rates showed that group C and D were significantly higher ($p < 0.05$) when compared with control group (Table 2). However, only success rate after 1st treatment by OTC was significantly higher as compared with control group. On the other hand, both plasma and Lotagen treated groups showed moderate improvement as success rates as well as pregnancy rates were not significantly different as compared with all treated groups. Moreover, the interval from calving to conception was significantly shorter by comparing treated groups C and D with treated groups A and B. However, interval from calving to conception for Lotagen treated group was significantly longer than OTC group ($p < 0.05$) and shorter than control and plasma treated groups (Table. 2).

In spite of that, calving condition did not have any significant effect on success rate after 1st treatment or overall success and pregnancy rates (45.6% vs.33.3%; 66.7% vs. 51.5% and 45.6% vs. 36.4%, respectively) but tended to increase for cows calved normally. However, the interval from calving to conception was significantly shorter (175.47 ± 5.19 vs. 191.33 ± 5.15 days, $p < 0.05$) when compared with cows with history of abnormal calving (Table 2).

Table 1: General information on treated groups and reproductive traits of animals included in this study

Treatment Group ¹	No. Prev. Calving*	Post partum days*
Saline (A) ²	3.27 ± 0.23	91.28 ± 2.43
Plasma (B)	3.06 ± 0.22	92.50 ± 2.80
Colostric serum (C)	3.11 ± 0.24	95.56 ± 3.30
Oxytetracycline (D)	3.39 ± 0.20	98.94 ± 2.16
Lotagen (E)	3.11 ± 0.24	97.28 ± 2.83

¹ 18 cows in each group were used., ² Control group. ^{*} Mean \pm S.E.

Table 2: Influence of treatment and calving condition on success rate; pregnancy rate and interval from calving to conception in dairy cows affected with endometritis.

Source of variation	Success rate after 1 st treat. ¹ (No)	Overall success rate ¹ (No)	Overall pregnancy rate ¹ (No)	Interval calving-conception*
<i>Treatment groups</i>				
(A)	22.2 (4) ^a	38.9 (7) ^a	16.7 (3) ^a	198.61± 6.30 ^b
(B)	27.8 (5) ^a	44.4 (8) ^{ab}	22.2 (4) ^a	195.78± 6.94 ^d
(C)	50.0 (9) ^{ab}	72.2 (13) ^b	55.5 (10) ^b	171.00± 9.58 ^{cd}
(D)	61.1 (11) ^b	83.3 (15) ^b	72.2 (13) ^b	157.72± 8.57 ^f
(E)	44.4 (8) ^{ab}	66.7 (12) ^{ab}	44.4 (8) ^{ab}	183.33± 8.22 ^{de}
<i>Calving condition</i>				
Normal (57)	45.6 (26) ^a	66.7 (38) ^a	45.6 (26) ^a	175.47± 5.19 ^d
Abnormal (33)	33.3 (11) ^a	51.5 (17) ^a	36.4 (12) ^a	191.33± 5.15 ^e

^{ab} Values in the same column with different superscripts are different (P < 0.05).

^{def} Means in the same column with different superscripts are different (P < 0.05).

1) in relation to the total number of animals. * Mean ± S.E.

Table 3: Analysis of variance of treatment and calving condition on interval from calving to conception in dairy cows affected with endometritis.

Source of variation	d.f	Interval calving – conception	
		M.S	F value
Treatment	4	7348.11	6.88**
Calving condition	1	8051.34	7.54**
Treat. X calving condition	4	2564.21	2.40 ^{ns}

** P < 0.01 ^{ns} non significant

DISCUSSION

It is generally accepted that the bovine uterus in the postpartum period is a septic environment, and the wide variety of microorganisms, which have been recovered, have been aptly referred to as facultative pathogens (Paisley *et al.*, 1986). In the current reproductive practice, it has been demonstrated that therapy of uterine infections ideally should

lead to exclusion of bacterial infection from the uterus without any suppression of the normal UDMs (Hussain and Daniel, 1991; Dhaliwal *et al.*, 2001).

Despite the extensive use of intrauterine antibiotics and antiseptics, bacterial endometritis is still the most common cause of bovine infertility (Bostedt, 1984; Olson, 1996). The moderate success rates achieved using antibiotics and antiseptics, in addition to the harmful effect on the uterine lining and economic loss incurred due to milk disposal directed research to alternative therapies utilizing natural substances that can increase leucocytic activity and UDM. In this study we compared two of the conventional intrauterine treatments; antibiotics (OTC) and antiseptics (Lotagen), immunostimulant; plasma and colostric serum and saline treatment for mild endometritis

The overall success rate of treatment was 61.1%, which tended to be lower than those reported (68%) by Sheldon and Noakes (1998). The clinical success of treatment is influenced by many factors eg. type and route of treatment, clinical severity, smell of vaginal discharge and the presence of corpus luteum (Murray *et al.*, 1990 Sheldon and Noakes, 1998). An important feature of bovine reproductive tract is effective self-cleansing mechanism, which is manifested by clearing bacterial infection (Griffin *et al.*, 1974). However, this property is not adequate in the face of *A. pyogenes* infection and high percentage of persistently infected cows are due to *A. pyogenes* (Bonnert *et al.*, 1991). In this study, spontaneous recovery was recorded in 7 cases (control group: 38.9%), which is in agreement with Steffan *et al.* (1984) who reported a 33% self-recovery rate. In a previous study, Al Dohni (2001) reported that *A. pyogenes* was isolated from 62 % of cows with endometritis. This might explain the high percentage of self-recovery failures in the control group of our study.

From the present investigation, it has been shown that the application of intrauterine infusion of OTC had higher success rates, pregnancy rate and shorter interval from calving to conception. Such results of effect of oxytetracycline are in agreement with those obtained from previous studies by Murray *et al.* (1990); Thurmond *et al.* (1993) and Sheldon and Noakes (1998). Although, OTC satisfies most of the criteria for the selection of antimicrobial substances for treatment of endometritis (Noakes, 1991); it is poorly absorbed from the uterus after an intrauterine infusion (Massera *et al.*, 1980) and its endometrial

concentrations are higher and persist longer than it has been administered by other parenteral routes (Anderson *et al.*, 1995). However, milk obtained from cows that had been treated by intrauterine infusion of OTC contained 316.9 micrograms/kg and should be discarded to avoid illegal residues (Dinsmore *et al.*, 1996). In addition, intrauterine application of antibiotics is more likely to accomplish levels sufficient to inhibit phagocytosis and other leukocyte activities (Jayappa and Luken, 1983).

Lotagen treatment produced clinical cure rates in 44.4% and 66.7% of cases after one and three treatments, respectively. Insemination of the successfully treated animals yielded pregnancy in 44.4% of cows, which tended to be different from the control group ($P > 0.05$). Lotagen is advocated as a treatment for endometritis in cattle (de Kruif *et al.*, 1982; Tenhagen and Heuwieser, 1999). It is claimed to cause coagulation in diseased and dead tissue, has bactericidal properties, does not affect healthy intact tissue and enhances the regeneration of a new endometrial epithelium. Although Lotagen is reported to induce moderate inflammatory and degenerative changes, and sloughing of the surface epithelium within days of installment, the regeneration time of the endometrium is completed within 7- 15 days or 35 day post (Schndyer *et al.*, 1990). This was reflected by acceptable success rate after first treatment (44.4%), which was not different from those obtained with OTC. The low pregnancy rate achieved after the first service was probably due to the short time between treatment and breeding. On the other hand, acceptable fertility rates were achieved when the endometrium was allowed sufficient time (2 cycles) to heal.

Antibody-mediated and cell-mediated immunity can be induced in the uterus after infection. (Wira *et al.*, 1999). The character and strength of a T-lymphocyte response is the net consequence of pivotal decision-making points in inductive effector pathways (Robertson, 2000). Local antibody production and phagocytosis via opsonins are important defense mechanisms in limiting uterine infection (Butt *et al.*, 1991). It was noticed that estrus phase associated with a mild cellular influx (neutrophils and leucocytes) into superficial endometrium (DeBois and Manspeaker, 1986). This occurred as a result of increased secretion of leukotierine B₄ a potent chemotactic molecule (Zerbe *et al.*, 1996), in inflamed uteri. Uterine infusion with cytokines (e.g. Interleukin) induce a significant increase in cellular influx and play

important roles as autocrine and paracrine immunoregulators (Belluzzi *et al.*, 1994). The present results revealed that intrauterine application of colostric serum improved the reproductive performance of dairy cows affected with endometritis and not different from those treated with OTC. Such results are not recorded before in the available literature. In the current study, colostric serum proved to have therapeutic effects in uterine infection which might be due to their high contents of immunoglobulins, complement, opsonins, cytokines e.g. interleukin and lysosome (Sordillo *et al.*, 1991; Jan, 1996). These components might have improved the phagocytic activity of endometrial leucocytes. Clover and Zarkower (1980) proved that factors stimulating the phagocytic activity of leucocytes in calves are transmitted with colostrum.

Previous study by Asbury (1984) suggested plasma infused intrauterine was expected to enhance the phagocytic activity of leucocytes through supplementation of plasma-derived opsonins to eliminate the uterine infection. The present study indicated that intrauterine infusion of plasma did not improve success rate (44.4%) and fertility (22.2%) of dairy cows affected with endometritis and was not different from control group and expected spontaneous recovery rates. The results of our study agree with those reported by Nanda *et al.* (1999). Furthermore, they suggested that plasma derived opsonins and the components of complement system transfused into the uterus are important up to some extent in cattle to exert a therapeutic effect in treating endometritis. The differences obtained in this study between the colostric serum and plasma treatment groups might be the results of the higher quantity of immunoglobulins, opsonins, cytokines and other unidentified factors in colostrum in comparison with plasma. Repeated daily plasma infusions have been successfully used to treat endometritis in mares. Further studies using higher doses of plasma (up to 100 ml every day for three successive days) are needed to evaluate its use in treating endometritis in cattle.

The mean interval from calving to conception of cows, which had assisted calving was significantly longer than that of normal animals and was associated with a non-significant reduction in the success and pregnancy rates. Murray *et al.*, (1990), observed a similar trend and Olson, (1996) who reported that dystocia and retained fetal membranes significantly reduce the success rates of treatment and prolongs the interval from calving to conception.

In conclusion, to our knowledge, this is the first report to describe the successful use of colostric serum in the treatment of endometritis in cattle. Although OTC demonstrated superior results in terms of success rate, pregnancy rate and days open, the differences were not significant. Economic losses due to cost and milk disposals may argue against the use of OTC and for the use of colostric serum in cases of mild endometritis.

REFERENCES

- Al-Dohni, S.N. (2001): Some studies on infectious causes of cattle infertility in Jordan. MSc. Thesis, Faculty of Graduate studies, Jordan University of Science and Technology.
- Anderson, K.L.; Moates, W.A.; Rushing, J.E.; Wesen, D.P. and Papich, M.G. (1995): Potential for oxytetracycline administration by three routes to cause milk residues in lactating cows, as detected by radioimmunoassay (charm II) and high-performance liquid chromatography test methods. *Am. J. Vet. Res.* 56 (1): 70-77.
- Asbury, A.C. (1984): Uterine defense mechanisms in the mare: The use of intrauterine plasma in the management of endometritis. *Theriogenology* 21 (2): 387-393.
- Belluzzi, S.M.; Galeotti, A.; Carluccio, A.; Matteuzzi, A; Boschi, S. and Trenti, F. (1994): The relationship between histology and chemical mediators in biopsy samples of endometrium from infertile cattle. *Proc. 18th World Buiatrics Cong., 26th cong. Italian Assoc. Buiatria, Bologna, Italy, Pp. 313-316*
- BonDurant, R.H. (1999): Inflammation in the bovine female reproductive tract. *J. Anim. Sci.* 77 Suppl 2/J: 101-109.
- Bonnett, B.N. and Martin, S.W. (1995): Path analysis of peripartum and postpartum events, rectal palpation findings, endometrial biopsy results and reproductive performance in Holstein-Friesian dairy cows. *Prev.Vet. Med.* 21: 279-288.
- Bonnet, B.N.; Martin, S.W.; Gannon, V.P.J.; Miller, R.B. and Etherington, W.G. (1991): Endometrial biopsy in Holstein-friesian dairy cows. III. Bacteriological analysis and correlations with histological findings. *Can. J. Vet. Res.* 55 : 168 -173.

- Bostedt, H. (1984): Uterine infections in the postpartum period. In Proceedings of the 10th International Congress on Animal Reproduction and AI. Urbana, Champaign, IV: III, pp.25-33.
- Butt, B.M.; Senger, P.L. and Widders, P.R. (1991): Neutrophil migration into the bovine uterine lumen following intrauterine inoculation with killed haemophilus somnus. *J. Reprod. Fertil.* 93: 341-345.
- Clover, C.K. and Zarkower, A. (1980): Immunological responses in colostrum-fed and colostrums deprived calves. *Am. J. Vet. Res.* 41: 1002 -1007.
- DeBois, C.H.W. and Manspeaker, J.E (1986): Endometrial biopsy of the bovine. *Current Therapy in theriogenology*. 2nd ed. D. Morrow, ed. W.B. Saunders Co. Philadelphia, PA. Pp 424-426 .
- De Kruif, A.; Gunnink, J.W. and de Bois, C.H. (1982): Diagnosis and treatment of postpartum endometritis in cattle. *Tijdschr Diergeneeskde*, 107 (19): 717 -725.
- Dhaliwal, G.S.; Murray, R.D. and Woldchiwet, Z. (2001): Some aspects of immunology of the bovine uterus related to treatment for endometritis. *Anim. Reprod. Sci.* 67: 135-152.
- Dhaliwal, G.S.; Singh, J. and Singh, N. (1998): Treatment of repeat breeding in dairy cows-a comprehensive approach. In: Proceedings of the 20th World Buiair Congress. Sydney. pp. 673-676.
- Dinsmore, R.P.; Stevens R.D.; Cattell, M.B.; Salman, M.D. and Sundlof, S.F. (1996): Oxytetracycline residues in milk after intrauterine treatment of cows with retained fetal membranes. *J.Am. Vet. Med. Assoc.* 209 (10): 1753 -1755.
- Griffin, J.F.T.; Hartigan, P.J. and Nunn, W.R. (1974): Non-specific uterine infection and bovine fertility. I. Infection patterns and endometritis during the first 7 weeks postpartum. *Theriogenology* (1) : 91-106.
- Gustafson, B.K. (1984): Therapeutic strategies involving anti-microbial treatment of the uterus of large animals. *J. Vet. Med. Assoc.* 185: 1194 - 1198.
- Hartigan, P.J.; Griffin, J.F.T. and Nunn, W.R. (1974): Some observations on corynebacterium pyogenes infection of the bovine uterus. *Theriogenology*. (1) : 153 -166.

- Hussain, A.M. and Daniel, R.C.W. (1991): Bovine endometritis: Current and future alternative therapy. *J. Vet. Med. A* 38: 641-651.
- Hussain, A.M.; Daniel, R.C.W. and O'Boyle, R.C.W. (1990): Postpartum uterine flora following normal and abnormal puerperium in cows. *Theriogenology* 34: 291-302.
- Jan, T. (1996): Mammary secretions and neonatal immunity. A review. *Vet. Res.* 27: 403 - 417.
- Jayappa, H. and Luken, K.I. (1983): Effect of antimicrobial agents and corticosteroids on bovine polymorphonuclear leucocytes chemotaxis. *Am. J. Vet. Res.* 44: 2155 -2159.
- Knutti, B; Kupfer, U. and Busato, A. (2000) Reproductive efficiency of cows with endometritis after treatment with intrauterine infusions or prostaglandin injections, or no treatment. *J. Vet. Med. A Physiol Pathol. Clin. Med.* 47 (10): 609 - 615.
- Massera, J.; Gustafsson, B.K.; Afiefy, M.M.; Stowe, C.M. and Bergt, G.P. (1980): Disposition of oxytetracycline in the bovine genital tract: systemic vs. intrauterine administration. *J. Am. Vet. Med. Assoc.* 176 : 1099 -1102.
- Murray, R.D.; Allison, J.D. and Gard, R.P. (1990): Bovine endometritis: comparative efficacy of alphaprostal and intrauterine therapies, and other factors influencing clinical success. *Vet. Rec.* 127: 86-90.
- Nakao, T.; Moriyoshi, M. and Kawata, K. (1988): Effect of postpartum intrauterine treatment with 2% polyvinyl-pyrrolidone-iodine solution on reproductive efficiency in cows. *Theriogenology* 30: 1033-1043.
- Nanda,P.S.A.; Grewal, A.S. and Singh, J. (1999): Uterine defense modulation for treatment of repeat breeding due to infectious endometritis in bovines. *Indian J. Anim. Sci.* 69 (5): 307 -309.
- Noakes, D.E. (1991): Cattle infertility. *Proceedings of British Cattle Veterinary Assoc.*, 1991-1992. p 47.
- Olson, J.D. (1996): Metritis/ endometritis: medically sound treatments. *Proc. of the 29th Ann Convent. of Am. Assoc. of Bovine Practitioners* . 29: 8-14.
- Paisley, L.G.; Mickelson, W.D. and Anderson, B.P. (1986): Mechanisms and therapy for retained fetal membranes and uterine infections of cows: review. *Theriogenology* 25 : 353 - 381.

- Parr, M.B. and Parr, E.L. (1999): Female genital tract immunity in animal models. In *Mucosal Immunity 2nd Ed.* Pp. 1395-1409. Eds Ogra P.I. Academic Press, San Diego.
- Robertson, S. A. (2000): Control of the immunological environment of the uterus. *Rev. Reprod.* 5: 164-174.
- Schnyder, D.; Kupfer, U. and Zwahlen, R. (1990): Changes in the endometrium of the cow after intrauterine administration of different drugs. *Schweiz Arch Tierheilkd* 132 (7): 353- 364.
- Sheldon, I.M. and Noakes, D.E. (1998): Comparison of three treatments for bovine endometritis. *Vet. Rec.* 142 : 575 -579.
- Singh, J.; Sidhu, S.S.; Dhaliwal, G.S.; Pangoonkar, G.R.; Nanda, A.S. and Grewal, A.S. (2000): Effectiveness of lipopolysaccharide as an intrauterine immune modulator in curing bacterial endometritis in repeat breeding crossbred cows. *Anim. Reprod. Sci.* 59: 159-166.
- Sordillo, L.M.; Redmond, M.J. and Campos, M. (1991): Cytokine activity in bovine mammary gland secretions during the periparturient period. *Can. J. Vet. Res.* 55: 298 -299.
- Sreenan, J. and Behan, D. (1974): Egg transfer in the cow: Pregnancy rate and egg survival. *J. Reprod. Fertil.* 41 (2): 497-499.
- Steffan, J.; Adriamanga, S. and Thibier, M. (1984): Treatment of metritis with antibiotics or prostaglandin F_{2α} and influence of ovarian cyclicity in dairy cows. *Am. J. Vet. Assoc.* 45: 1090-1093.
- Subandrio, A.L. and Noakes, D.E. (1997): Neutrophil migration into the uterine lumen of the cow: the influence of endogenous and exogenous sex steroid hormones using two intrauterine chemo attractants. *Theriogenology* 47: 825-835.
- Tenhagen, B.A. and Heuwieser, W. (1999): Comparison of a conventional reproductive management programme based on rectal palpation and uterine treatment of endometritis with a strategic prostaglandin F_{2α} programme. *Zentralbl Veterinarmed A*, 46 (3): 167 -176.
- Thurmond, M.C.; Jameson, C.M. and Picanso, J.P. (1993): Effect of intrauterine antimicrobial treatment in reducing calving-to-conception interval in cows with endometritis. *J. Am. Vet. Assoc.* 203 (11): 1576-1578.