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Research Article

The Effect of Dinoprost Treatment on *Trueperella pyogenes* in Uterus of Cows with Pyomtra

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Abstract: To investigate the effect of dinoprost to the exclusion of the purulent exudate, the change of intrauterine bacteria and the reproductive performance in cows with pyometra, 13 Holstein cows with pyometra were treated with tromethamine dinoprost intramuscularly (25 mg as dinoprost) one time on the day when pyometra was diagnosed. Then intrauterine perfusion fluid was collected for bacteriological examination at 3 days and 21 days after dinoprost treatment. In all cows estrus was induced by the dinoprost treatment within four days and the purulent material in the uterus was excreted from the uterus. Although *Trueperella pyogenes* was isolated in 12 cows out of 13 cows at the first bacteriological examination, it was isolated in only two cows at the second bacteriological examination. Seven out of 10 cows which received artificial insemination became pregnant after the dinoprost treatment. In conclusion, the dinoprost treatment is effective to not only exclude the purulent exudate but also eliminate *T. pyogenes* from uterus

Keywords: Cattle, dinoprost, pyometra, *Trueperella pyogenes*, uterus

INTRODUCTION

Pyometra is a condition which occurs frequently in cows during the postpartum period and it is defined by the accumulation of purulent material within the uterine lumen, the presence of a persistent Corpus Luteum (CL) and a closed cervix (Mortimer *et al*., 1993; Sheldon *et al*., 2006). *Trueperella pyogenes* (*T. pyogenes*) is usually associated with that condition. Uterine infections in cattle and its pathogenicity to uterus are reinforced by *Escherichia coli* (*E.coli*) and gram-negative anaerobic bacteria like *Fusobacterium necrophorum* (Farin *et al*., 1989; Olson *et al*., 1984). As the results, cows show anestrous and reproductive efficiency is reduced. The treatment method for pyometra was administration of estrogen product and it merely aimed to exudate content of uterus by relaxation of cervix (Roberts, 1971) and then the method was replaced by administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) product (Risco *et al*., 2007). However the report about the treatment results, the change of intrauterine bacteria and the reproductive performance after the $PGF_{2\alpha}$ administration are few. We investigated the effect of the $PGF_{2\alpha}$ administration to bovine pyometra in this study.

MATERIALS AND METHODS

Cows: Thirteen Holstein cows with pyometra were used. The mean number of days from the parturition to the diagnosis of pyometra was 79.7±31.8 days (mean±SEM) (Table 1). The pyometra was suspected based on the following signs, no Artificial Insemination (AI) history, anestrus, the existence of Corpus Luteum (CL) and the distention of uterus which was similar to a pregnant uterus. Then the existence of echogenic purulent material in the uterus was clarified with transrectal real-time ultrasonography (Model HS-2100 V; Honda Electronics Co., Ltd, Aichi, Japan) equipped with a 10-MHz transrectal linear-array transducer. As a dinoprost treatment, all cows received tromethamine dinoprost (Pronalgon F, Pfizer, Tokyo, Japan) intramuscularly (25 mg as dinoprost) one time on the day when pyometra was diagnosed.

Intrauterine perfusion fluid collection: The intrauterine perfusion fluid was collected by method of Kaneko *et al*. (1996) from all cows for bacteriological examination at 3 days and 21 days after dinoprost treatment. A vaginal speculum was inserted into the vagina after cleansing of the vulva with a disinfectant and the tip of a balloon catheter (Terumo inc., Tokyo, Japan, Fr. 22) was inserted into the cervix as deep as possible without touching the vaginal wall. Then the vaginal speculum was removed, the balloon catheter was advanced into the uterus using the recto-vaginal method and the balloon was inflated. Sterile physiological saline (100 mL) was infused into the uterus through a balloon catheter and recovered by gently massaging the uterus.

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Table 1: Bacteriological findings and reproductive performance

Cow no.	First examination Bacteria species	Second examination Bacteria species	AI	Conception	Perturition to treatment	Perturition to conception	Treatment to conception	AI number
1	*T. pyogenes* *E. coli*	N	-		79			
2	*T. pyogenes*	N	+	+	157	231	174	2
3	*T. pyogenes*	N	-		80			
4	*T. pyogenes*	*T. pyogenes*	+	-	69			Infertile
5	*T. pyogenes*	N	+	-	45			Infertile
6	*T. pyogenes*	*E. coli*	+	-	91			Infertile
7	*T. pyogenes*	N	+	+	133	225	92	3
8	*T. pyogenes* *Proteus spp.*	*T. pyogenes*	+	+	59	107	48	1
9	*T. pyogenes* *Proteus spp.*	N	+	+	58	159	101	3
10	*T. pyogenes*	N	+	+	67	216	149	4
11	*N*	N	+	+	78	152	74	3
12	*T. pyogenes*	N	-		61			
13	*T. pyogenes*	N	+	+	59	99	40	2
Mean±SEM					79.7±8.8	169.9±20.9	96.7±18.8	2.6±0.4

T. pyogenes, *Trueperella pyogenes*; *E. coli*, *Escherichia coli*; N, not detected; AI, Artificial insemination; Treatment, dinoprost treatment; Infertile, Slaughtered due to infertility

Bacteriological examination of intrauterine perfusion fluid: The perfusion fluid (10 mL) was centrifuged at 1,000×g for 10 min and the sediment was resuspended in 1 mL of physiological saline after removal of the supernatant. An aliquot of the resuspended sediment (100 μL) was applied to soy agar with 5% sheep blood and incubated aerobically at 37°C for 48 h. Using the criteria of Kaneko *et al*. (1996), samples showing growth of more than 10 identical colonies were defined as positive for bacteria. Gram-negative, atypical, pine leaf-like rods, which showed hemolytic reaction on sheep blood-containing agar medium and were negative to the catalase test, were judged to be *T. pyogenes*.

Reproductive performance: The number of days from the parturition to the dinoprost treatment, the number of days from parturition to conception, the number of days from the dinoprost treatment to conception and the number of Artificial Inseminations (AI) required to conception were monitored.

Statistical analysis: The mean number of days from parturition to the dinoprost treatment was compared between two groups (concept group and not concept group) by Student's *t*-test.

RESULTS

Bacteria in the intrauterine perfusion fluid: In all cows, estrus was induced by the dinoprost treatmen within four days and the purulent material in the uterus was excreted from the uterus. The result of the bacteriological examination was shown in Table 1**.** *T. pyogenes* was isolated in 12 out of 13 cows at the first bacteriological examination and any bacterium was isolated in one cow. *Proteus* spp. was isolated in two cows and *Escherichia coli* (*E. coli*) was isolated in one cow out of 12 cows from which *T. pyogenes* was isolated (Table 1). However *T. pyogenes* was isolated in only two cows at the second bacteriological examination and *Proteus* spp. and *E. coli* which was isolated at the first bacteriological examination disappeared at this time.

Reproductive performance: Seven out of 10 cows which received AI became pregnant (concept group) and three cows were slaughtered due to infertility (not concept group). Other three cows were slaughtered due to other reasons. The mean number of days from parturition to the dinoprost treatment for all cows, the concept group and the not concept group was 79.7±8.8, 87.3±15.3 and 68.3±8.7, respectably and there was no significant difference between the concept group and the not concept group. The mean number of days from parturition to conception was 169.9±20.9, the mean number of days from the dinoprost treatment to conception was 96.7±18.8 and the mean AI number required conception was 2.6±0.4 in the concept group (Table 1).

DISCUSSION

The bovine pyometra develops after the first ovulation in presence of an active luteal tissue, usually from about the 20th-21st day onwards and cows show anestrus due to the persisting luteal tissue (Földi *et al*., 2006). The mean number of days from parturition to the dinoprost treatment for all cows was 79.7±31.8 (mean±SEM). This means that a farmer notices pyometra because cows do not show estrus at the time when they need to be inseminated.

Although *T. pyogenes* was isolated from the uterus in 12 out of 13 cows three day after the dinoprost treatment, only two cows had maintained *T. pyogenes* in their uterus 18 days later. This shows that the

dinoprost treatment can not only exclude the purulent exudate but also eliminate *T. pyogenes* from uterus effectively. In pyometra, progesterone has a role to maintain functional closure of the cervix as well as increasing the susceptibity to persistent infection (Noakes *et al*., 1990). The effect of the dinoprost treatment might be attributable to the luteolytic effect, which induces estrus and causes estrus mucus to flush the endometrium and help in rapid care (El-Tahawy and Fahmy, 2011). Furthermore, exogenous $PGF_{2\alpha}$ stimulates uterine production of $PGF_{2\alpha}$ and allows the uterus to resolve *T. pyogenes* infection, even when progesterone is maintained at luteal phase concentration before and after treatment (Gregory, 2003). $PGF_{2\alpha}$ is a proinflammatory molecule that stimulates the production of various proinflammatory cytokines and it may enhance uterine production of leukotriene B_4. Proinflammatory cytokines and leukotriene B_4 enhance phagocytosis and lymphocyte function (Gregory, 2003). Even though there are clear association among $PGF_{2\alpha}$, leukotriene B_4, proinflammatory cytokines phagocytosis and lymphocyte functions, the mechanism of action of exogenous $PGF_{2\alpha}$ in overriding the down-regulatory effects of progesterone and resolving uterine infections has not been elucidated (Gregory, 2003). Defining this mechanism should yield new prevention and treatment strategies for uterine infections that do not rely on antibiotic and antimicrobial compounds (Gregory, 2003).

Isolation of *T. pyogenes* at the late involution period (28~35 days after calving) is associated with dramatically decreased of re-conception rate (Huszenicza *et al*., 1999). Endometrial damage corresponded with the length of time during which *T. pyogenes* was positive and the duration of the infection determined the effect on subsequent fertility (Hartigan *et al*., 1974). However there was no significant difference between the concept group and the not concept group in the number of days from parturition to the dinoprost treatment. *T. pyogenes* lacks the ability to invade intact epithelium and requires a damaged epithelium by *E. coli* and gram-negative anaerobic bacteria to establish infection (Dohmen *et al*., 2000). Therefor, there may be the difference of the degree of tissue damage among individuals even if *T. pyogenes* exists in their uterus. In chronic forms of clinical endometritis the scar tissue may replace the functional endometrium, resulting in periglandular fibrosis, cystic degeneration and/or atrophy of uterine glands (Lewis, 1997). Although we had not performed endometrila biopsy in this study, there might be difference in the degree of histological changes among individuals even if the purulent exudate accumulates in the uterus.

It is unknown whether CL persistency is responsible for the pyometra, or is a consequence of it and what the real underlying mechanism is Földi *et al*. (2006). However the dinoprost treatment excluded the purulent exudate and eliminated *T. pyogenes* from uterus. Furthermore seven cows out of 10 which were given AI conceived after the dinoprost treatment. These results indicate that the dinoprost treatment is effective method to treat bovine pyometra.

REFERENCES

Dohmen, M.J.W., K. Joop, A. Sturk, P.E.J. Bols and J.A.C.M. Lohuis, 2000. Relationship between intra-uterine bacteria concentration, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. Theriogenology, 54: 1019-1032.

El-Tahawy, A.S. and M.M. Fahmy, 2011. Partial budgeting assessment of the treatment of pyometra, follicular cysts and ovarian inactivity causing postpartum anoestrus in dairy cattle. Res. Vet. Sci., 90: 44-50.

Farin, P.W., L. Ball, J.D. Olson, R.G. Mortimer, R.L. Jones, W.S. Adney and A.E. McChesney, 1989. Effect of Actinomyces pyogenes and gram-negative anaerobic bacteria on the development of bovine pyometra. Theriogenology, 31: 979-989.

Földi, J., M. Kulcsár, A. PéCsi, B. Huyghe, C. De Sa, J.A.C.M. Lohuis, P. Cox and Gy. Huszenicza, 2006. Bacterial complications of postpartum uterine involtion in cattle. Anim. Reprod. Sci., 96: 265-281.

Gregory, S.L., 2003. Steroidal regulation of uterine resistance to bacteria infection in livestock. Reprod. Biol. Endocrinol., 1: 117-124.

Hartigan, P.J., J.F.T. Griffin and W.R. Nunn, 1974. Some observation on Corynebacterium pyogenes infection of the bovine uterus. Theriogenology, 1: 153-167.

Huszenicza, G.Y., M. Fodor, M. Gacs, M. Kulcsar, M.J.V. Dohmen, M. Vamos, L. Porkolab, T. Kegl, J. Bartyik, J.A.C.M. Lohuis, S.Z. Janosi and G. Szita, 1999. Uterine bacteriology, resumption of cyclic ovarion activity and fertility in postpartum cows kept in large-scale dairy herds. Reprod. Domest. Anim, 34: 237-245.

Kaneko, K., S. Kawakami, M. Miyoshi, T. Abukawa, S. Yamanaka, M. Mochizuki and S. Yoshihara, 1996. Bacteriological and cytological examination of the uterine perfusate in cows. J. Jpn. Vet. Med. Assoc., 49: 435-438.

Lewis, G.S., 1997. Uterine health and disorders. J. Dairy Sci., 80: 984-994.

Mortimer, R.G., J.D. Olson, E.M. Huffman, P.W. Farin, L. Ball and B. Abbitt, 1993. Serum progesterone concentration in pyometritic and normal postpartum dairy cows. Theriogenology, 19: 647-653.

Noakes, D.E., L.M. Wallace and G.R. Smith, 1990. Pyomtra in a Friesian heifer: Bacteriological and endometrial changes. Vet. Rec., 126: 509.

Olson, J.D., L. Ball, R.G. Mortimer, P.W. Farin, W.S. Adney and E.M. Huffman, 1984. Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. Am. Vet. Res., 45: 2251-2255.

Risco, C.A., R.S. Youngquist and M.D. Shore, 2007. Postpartum Uterine Infections. In: Current Therapy in Large Animal Theriogenology. 2nd Edn., Saunders Elsevier Missouri, St. Louis, Mo, pp: 1088, ISBN: 1437713408.

Roberts, S.J., 1971. Other Pathological Causes for Bovine Infertility. In: Veterinary Obstetrics and Genital Diseases. 2nd Edn., Comstock, Ithaca, New York, pp: 471-496.

Sheldon, I.M., G. Lewis, S. LeBlanc and R. Gilbert, 2006. Defining postpartum uterine disease in dairy cattle. Theriogenology, 65: 1516-1530.