Recent Possibilities for Diagnosis and Treatment of Post Parturient Uterine Diseases in Dairy Cow

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Abstract

The successful genetic selection for higher milk production caused a dramatic decline in the reproductive performance of dairy cows all over the world during the last decades. Achievement of optimum herd reproductive performance (calving interval of 12 months or 13 months with the first calf born at 24 months of age) requires concentrated management activities especially during the first 100 days following calving. The following management activities are needed to pursue during the early postpartum period to reach or approach the optimal reproductive performance such as careful surveillance and assistance at calving, prevention of post parturient metabolic diseases, early diagnosis and treatment of post parturient uterine diseases, accurate detection of oestrus, correct timing of insemination, reducing the effect of heat stress and early pregnancy diagnosis. Among these main activities only early diagnosis and treatment of post parturient uterine diseases and their effects on reproductive performance are discussed. Clinical metritis and clinical endometritis (or recently used purulent vaginal discharge: PVD) can be accurately diagnosed in the field, however it is very important to remark that the presence of PVD alone is not sufficient to diagnose clinical endometritis because cervicitis and/or vaginitis can also cause PVD. Both diseases have detrimental effect on reproduction therefore it is important to diagnose as soon as possible and treat accordingly.

Keywords: Dairy cow; Clinical metritis; Clinical endometritis; Subclinical endometritis

The successful genetic selection for higher milk production in Holstein cows has nearly doubled the average milk production in the United States since 1960, to over 11,000 kg/year. Over the same time period, there has been a dramatic decline in the reproductive performance of dairy cows. The average number of days open (interval from calving to conception) and the number of services per conception have increased substantially. In order to decrease the longer lactations and the number of cows culled for reproductive reasons it is very important to improve our reproductive management practices [1]. Achievement of optimum herd reproductive performance (calving interval of 12 months or 13 months with the first calf born at 24 months of age) requires concentrated management activities especially during the first 100 days following calving. Early postpartum breeding of dairy cows results in more calves, and higher milk production per lactation [2]. Poor reproductive performance can reduce the number of calves born and milk production and may increase the cost of therapy and semen.

The following management activities such as careful surveillance and assistance at calving, prevention of post parturient metabolic diseases, early diagnosis and treatment of post parturient uterine diseases, accurate detection of oestrus, correct timing of insemination, reducing the effect of summer heat stress and early pregnancy diagnosis are needed to pursue during the early postpartum period to reach or approach the optimal calving interval [3].

Among these main activities only early diagnosis and treatment of post parturient uterine diseases and their effects on milk production and reproductive performance are discussed in the present work. However, this topic has also a great importance because it is generally accepted that up to 40% of dairy cows may have clinical metritis within the first two weeks after calving and infection may persist in 10 to 15% of animals more than 3 weeks after calving causing clinical or subclinical endometritis [4].

Clinical Metritis

Clinical metritis is an acute systemic illness due to infection of the uterus with bacteria, usually within 10 (21) days after parturition.

According to Sheldon et al. [5], clinical metritis can be categorized into three grades:

Grade 1 clinical metritis (CM1) can be characterized by an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days after calving.

Grade 2 clinical metritis (CM2) or puerperal metritis can be characterized by a fetid red-brown watery uterine discharge, atomic enlarged uterus and, usually pyrexia (>39.5°C) [6,7]; in severe cases, reduced milk yield, dullness, inappetence or anorexia, elevated heart rate, and apparent dehydration may also be present. In some cases pyrexia even with daily monitoring of rectal temperature could not be detected [4,8] however an enlarged uterus with a thin wall and atonia used to be present with a fetid discharge. Puerperal metritis is often associated with retained placenta, dystocia, stillbirth or twins, and usually occurs toward the end of the first week after calving, being rare after the second week after calving [7,9,10]. It is important to emphasize

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Received December 07, 2015; Accepted February 03, 2016; Published February 10, 2016

Citation: Szenci O (2016) Recent Possibilities for Diagnosis and Treatment of Post Parturient Uterine Diseases in Dairy Cow. JFIV Reprod Med Genet 4: 170. doi:10.4172/2375-4508.1000170

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that puerperal metritis (CM2) may occur in around 10%-15% of cows with spontaneous calving and without retained foetal membranes [8].

Grade 3 clinical metritis (CM3) or toxicaemic metritis can be characterized by additional signs of toxemia (such as cold extremities, depression and/or collapse) which has a poor prognosis [5].

Diagnosis of clinical metritis

Diagnosis of clinical metritis (CM1 to CM3) is based on the clinical signs (purulent uterine discharge within 21 days after calving (CM1), fetid watery red-brown uterine discharge, fever >39.5°C, dullness and decreased milk yield (CM2), additional signs of toxemia such as cold extremities, depression and/or collapse (CM3) and abnormally enlarged uterus detected by rectal palpation mainly during the first week (10 days) after calving and rare during the second week [6,10].

Diagnosis of clinical metritis in small farms is based on the presence of vaginal discharge because clinical symptoms are not characteristics. It is important to mention that vaginal discharge can be present only in 24-33% of CM2 cases [11] and this may explain why the farmers used to present CM2 only about 2% of calvings [12] instead of 10% when systematic examination (e.g. measuring rectal temperature) is used [6,13].

In contrast, to diagnose it in large herds may be more complicated. Measuring the body temperature daily during the first 10 days after calving and performing vaginal examination at least once between Days 2 to 10 may help diagnosing clinical metritis more accurately. Measuring only rectal temperature is not enough because some cows may have no elevated rectal temperature (>39.5°C) and have CM2 puerperal metritis [4,8]. The rectal measures of body temperature can be influenced “by the procedure itself (up to 0.5°C), type of thermometer (up to 0.3°C), and the penetration depth into the rectum (up to 0.4°C difference between a penetration depth of 11.5 cm and 6.0 cm in one of the experiments). Differences in rectal temperature before and after defecation are minor (<0.1°C). These results may indicate that some care is required in generalizing rectal measures of body temperature” [14]. Daily measurement of rectal temperature and the assessment of vaginal discharge (colour, smell and viscosity) may help the diagnostic accuracy of puerperal metritis [15]. Using an electronic nose (DiagNose, C-it, Zutphen, the Netherlands) may increase the accuracy for evaluating vaginal discharge odour however the system needs further development for the field use [16].

It is important to emphasize that this early vaginal examination must perform with great care using adequate lubricant and being as hygienic as possible. The accuracy of our diagnosis can be improved by monitoring milk yield because milk production in some cows is not increasing daily as expected after calving or there is a sudden drop in it. Reduced feeding activity during the pre-partum period can be a significant risk factor for developing puerperal metritis [17]. In contrast, only a moderate accuracy can be reached by measuring the serum concentrations of acute phase proteins (haptoglobin) in dairy cows (sensitivity: 50% to 79%, specificity: 54% to 87%, respectively) [18,19].

Treatment of clinical metritis

Early treatment of clinical metritis (especially puerperal metritis) may decrease the severity of genital disorders (endometritis, cystic ovarian disease), the predisposition of metabolic disorders (left displacement abomasums, ketosis) and other complications like pyelonephritis, arthritis, endocarditis, hepatic and pulmonary abscesses [20-24].

There are a great variety of treatment protocols such as intrauterine antimicrobial agents (oxtetracycline; ampicillin and cloxacillin), antiseptic chemicals (iodine solutions: 500 ml of 2% Lugol’s iodine immediately after calving and again 6 hr later as a preventive measure or 200 ml of povidone iodine diluted in 2 L of distilled water [25]), organic certified product (Optimum UterFlush, Van Beek Natural Science, Orange City, IA) in organic dairy farms [26], systemic antibiotics (penicillin or one of its synthetic analogues/amoxicillin) [26]; ampicillin, oxytetracycline, cefotiof /third generation cephalosporin/: 2.2 mg/kg of body weight daily for 3 to 5 days; 2 doses of 6.6 mg cefiofur crystalline free acid sterile suspension (CCFA SS)/kg of body weight s.c. in the base of the ear at a 72 hr interval [27] or a single dose of CCFA-SS within 24 hr after calving as a preventive measure [28,29], ozone i.u. treatment [30], supportive therapy (nonsteroidal anti-inflammatory drugs such as flunixin meglumine [31], fluid therapy in case of dehydration, therapy with calcium and energy supplements in case of depressed appetite, and hormone therapy (oxytocin: 20 to 40 IU repeated every 3 hr to 6 hr within 48 hr to 72 hr after calving; PGF$_{2a}$ or its synthetic analogues) have been introduced in the field [32]. The prognosis for recovery from puerperal metritis (CM2) varies with severity of the condition.

According to our present knowledge intrauterine antimicrobial and antiseptic treatments cannot be recommended because of irritating the endometrium [32]. Routine use of hormone therapies (PGF$_{2a}$) is also controversial and needs further confirmations. It seems that presently systemic antibiotic (cefotiofur) and supportive therapy can be recommended for the field [6,33]. Reviewing 21 current literature data dealing with the treatment of puerperal metritis, Haimerl and Heuwieser [34] found that most of the studies used cefotiofur (17 of 21) and only 7 studies observed clinical improvement and none of them found improved reproductive performance. It is also important to mention that there is a growing concern of using third and fourth generation cephalosporins in production animals [35] therefore as an alternative therapy amoxicillin (i.m.) and intrauterine oxytetracycline infusion [26] or intrauterine ozone therapy can be recommended [36]. Due to the prevalence of self-recovery of puerperal metritis antibiotic treatment protocol can be started on Day 5 after calving however this hypothesis needs to be confirmed in a field study [37].

Treatment of acute puerperal metritis with a single dose of flunixin meglumine in addition to antibiotic treatment had no beneficial effect on clinical cure, milk yield within 6 d after the first treatment, or reproductive performance [31,38], while according to Amiridis et al. [39], a single dose of flunixin meglumine (2.2 mg/kg BW) administered intravenously to cows with CM2 between Days 5 and 8 after calving accelerated the uterine involution and shortened the calving-to-first-estrus interval.

Retained Placenta

Retained placenta or retained foetal membranes may occur if the placenta has not been shed by 12 (24) hr after calving. Majority of the placenta used to be expelled within 6 hr-9 hr after calving (88.7%) [40]. The average incidence of retained placenta after normal calving used to be 7% between 3 (4) to 11 (12%) [41,42]. After abnormal delivery (e.g., twin pregnancy, Caesarean section, fetotomy, forced extraction of the foetus, abortion, premature calving) and in herds infected with brucellosis its incidence rate can range between 20 and 50% or even more. Several factors like genetic, nutritional, immunological and pathological ones may influence the separation of bovine placenta; however its aetiology is still not fully understood. As retained placenta predisposes the development of uterine infections (clinical metritis, as well as clinical and subclinical endometritis) [43], and causing a

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decrease in milk production (decreased milk yield, milk from treated cows withheld) [4,44] and reproductive performance (increases in days open, services per conception, calving to first heat interval, days from calving to first service and culling rate) [45] therefore the aim of its therapy is to prevent its adverse side effects.

Treatment of retained placenta

The aim of the treatment for retained placenta is to reduce the occurrence of puerperal metritis and subsequently clinical and subclinical endometritis, to decrease milk losses, to reduce reproductive inefficiency, and to decrease veterinary expenses [9,44].

There are a great variety of treatment protocols (manual removal of retained membranes, intrauterine treatments with antibiotics or antiinfectives /Lugol’s iodine/ [46], ozone spray [47,48], hormones /oxytocin, prostaglandins/, ergot derivatives, calcium, injection of collagenase into the umbilical arteries, versus no treatment) recommended for the field [49]. However, all of these methods have some limited values in the treatment of retained placenta [41]. Recent findings confirm that systemic antibiotics (ceftiofur 1 mg/kg) without intrauterine manipulation and treatment can be as effective as conventional treatment [6]. This was also confirmed in a later study in febrile cows [50,51]. It seems that systemic antibiotic is effective if the selection of treatment based on fever which may reduce the use of antibiotics compared with intra-uterine antibiotics [51]. Treatment with oxytocin, PGF$_{2\alpha}$ or calcium was not effective for the prevention of retained placenta [52] or did not hasten the passage of foetal membranes [53] while injection with a new Chinese herbal medicine (40 mL of Dang Hong Fu) into the standard acupoint GV-1 (located in the depression halfway between the anus and the ventral aspect of the coccygeal vertebrae) decreased the prevalence of retained placenta and the time until expulsion of foetal membranes [54].

Clinical endometritis

Clinical endometritis is characterized by the presence of purulent or mucopurulent uterine discharge detectable in the vagina, 21 days or more after calving, and is not accompanied by systemic signs [45,55]. Due to the fact that mostly there is no endometrial inflammation (endometritis) in case of purulent vaginal discharge (PVD) therefore according to Dubuc et al. [56] the PVD terminology should be used.

Diagnosis of clinical endometritis

There are several methods (transrectal palpation [45]), transrectal ultrasonography [57], histological examination of endometrial biopsies [5,58], manual vaginal examination [5], vaginoscopy [45] available in the field to diagnose clinical endometritis in the cow however each method has some limitations. In a recent study it was confirmed that vaginoscopy was a practical tool to distinguish healthy from diseased cows with clinical endometritis [59].

In contrast, Metricheck® (Simcro, New Zealand) consisting of a stainless steel rod with a rubber hemisphere can be used to retrieve vaginal contents more easily and precisely. In a recent study three methods (vaginoscopy /reference method/, gloved hand and Metricheck) were compared for diagnosing clinical endometritis between Days 21 to 27 after calving and it was confirmed that somewhat more cows (47.5%) could be diagnosed by Metricheck than by the other two methods (vaginoscopy: 36.9%, gloved hand: 36.8%). At the same time it did not result in improved reproductive performance [60]. On the other hand cytobrush cytology is also a reliable method for diagnosing clinical endometritis in cattle [61] however it is not so practical in the field.

The character and the odour of the vaginal mucus [4] can be scored according to the followings:

**Mucus character**
- Score 0: clear or translucent mucus;
- Score 1: mucus containing flecks of white or off-white pus;
- Score 2: discharge containing ≥ 50% white or off-white mucopurulent material;
- Score 3: discharge containing ≥ 50% purulent material, usually white or yellow, but occasionally sanguineous.

**Mucus odour**
- Score 0: no unpleasant odour
- Score 3: Fetid odour

In the absence of a gold standard it seems that vaginoscopy or Metricheck is preferred to use as a cow-side diagnostic tool for diagnosing clinical endometritis in the field [62].

Treatment of clinical endometritis

The general principle of the treatment of clinical endometritis is to reduce the load of pathogenic bacteria and enhance uterine defence and repair mechanisms and hence halt and reverse inflammatory changes that impair fertility [63]. A wide variety of therapy has been reported for clinical endometritis, including systemically or locally administered antibiotics, locally administered antiinfective solution and/or systemically injected PGF$_{2\alpha}$. Infusion of antimicrobials into the uterus is aimed at achieving high concentrations at the site of infection [64,65]. In contrast to systemic administration, intrauterine administration achieves higher drug concentration in the endometrium, but little penetration to deeper layers of the uterus or other genital tissues.

Intrauterine treatment with 0.5 g cepahiprin, first-generation cephalosporin, at 24-42 days before the planned start of mating improved reproductive performance of dairy cattle, especially those that had a history of retained placenta, a calf dead at calving or within 24 hr of calving, or vulval discharge [66]. In an experimental study, systemic administration of cequinome (1 mg/kg), fourth generation cephalosporin, for three consecutive days was efficient for treatment E. coli-induced endometritis [67]. Intrauterine infusion of cepahiprin or systemic administration of PGF$_{2\alpha}$ significantly improved the pregnancy rate of cows with clinical endometritis from which T. pyogenes was isolated [68]. A field study revealed that odd ratios for pregnancy after treatment of cows with clinical endometritis with PGF$_{2\alpha}$ or cepahiprin on Days 28 to 35 postpartum were 1.5 and 1.9 (P<0.05), respectively as compared to the control [33]. In a large field study performed by LeBlanc et al. [45,69], there was no benefit on time to pregnancy of treatment of endometritis before 4 week postpartum. Moreover, administration of PGF$_{2\alpha}$ between 20 and 26 DIM to cows with endometritis that did not have a palpable CL was associated with a significant reduction in pregnancy rate. Cows with endometritis between 27 and 33 DIM, treated with cepahiprin i.u. had a significantly shorter time to pregnancy than cows in the untreated groups. In cows with endometritis that had a palpable CL, there was no significant difference in time to pregnancy between those treated by intrauterine infusion of cepahiprin or PGF$_{2\alpha}$. Both groups tended to have a higher pregnancy rate than those in the untreated cows. Numerous reviewers have concluded that PGF$_{2\alpha}$ appears to be at least as effective for clinical endometritis as any available alternative therapy (Lugol’s iodine [68], polyvinylpyrrolidone-iodine solution [70], metacresolsulphuric acid

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and Lotagen [71]) and presents minimal risk of harm to the uterus or presence of residues in milk or meat [64,72,73].

In the absence of an active corpus luteum, the treatment efficacy of clinical endometritis with only prostaglandin injection is limited however such a treatment according to Lewis [74] may bring certain advantages as well.

It is important to mention that cows with clinical endometritis treated with one or 2 PGF$_{2\alpha}$ before initiation of the timed AI program had the lowest pregnancy rate per AI and the highest pregnancy loss compared with those having no uterine diseases [75]. A recent meta-analysis was also not able to confirm the benefit of PGF$_{2\alpha}$ treatment on reproductive performance of dairy cows with endometritis [76]. Similarly, 2 PGF$_{2\alpha}$ treatments between Days 37 ± 3 and 51 ± 3 after calving as part of an oestrous synchronization protocol and i.u. infusion of cefotiofur (125 mg) given at Day 44 ± 3 did not improve pregnancy per AI following the first postpartum insemination or the rate of pregnancy in the first 300 after calving [77].

Recently, as an alternative treatment of antimicrobials for clinical endometritis, 50% Dextrose [78] or proteolytic enzyme (containing trypsin, chymotrypsin and papain) solutions [79] were used however further investigations are needed to confirm the beneficial effects of such treatments [80].

**Pyometra**

Pyometra is characterized by the accumulation of purulent or mucopurulent material within the uterine lumen, distension of the uterus in the presence of an active corpus luteum and a closed cervix [5]. There are often an increased number of pathogenic bacteria within the uterine lumen when the corpus luteum forms and pyometra occurs [81]. Although there is a functional closure of the cervix, the lumen is not always completely occluded and some pus may be discharged through the cervix into the vaginal lumen.

The diagnosis of pyometra can be based on transrectal palpation of a distended uterus and/or on transrectal ultrasonography of mixed echodensity fluid and the presence of a persistent corpus luteum, with a history of anoestrus [5].

**Treatment of pyometra**

The best treatment protocol is to use prostaglandin (PGF$_{2\alpha}$ or its synthetic analogues) injection(s) because of the presence of a persistent corpus luteum. Due to common relapse it is recommended to repeat the prostaglandin treatment 12 to 14 days later. Intra-uterine antibiotic therapy (cephapirin) may be used as well. Complete restoration of the endometrium may need 4 to 8 weeks therefore it is very important to diagnose and treat pyometra as soon as possible after calving to decrease the destructive nature of pyometra on the endometrium [73].

**Subclinical Endometritis**

Subclinical endometritis can be diagnosed by uterine cytology in the absence of purulent discharge in the vagina. Endometrial and inflammatory cells may be collected by uterine lavage [82,83], cytobrush [82,83] or cytotype techniques [84] to evaluate the presence of PMNs in the uterine sample. If >18% PMNs in uterine cytology samples collected between Days 21 and 33 after calving, or >10% PMNs at Days 34 to 47 after calving can be found, in the absence of clinical endometritis, subclinical endometritis can be defined [82]. If >5% PMNs in uterine cytology samples collected by uterine lavage between Days 40 and 60 after calving can be found, subclinical endometritis is defined [85]. It is important to mention that there are a wide range of cut-off values to diagnose subclinical endometritis in the field [62,86,87].

By comparing the cytobrush technique, cytotape provided higher quality cells and significantly less blood contaminations while the PMNs percentage and the total number of cells did not differ [84].

Subclinical (cytologic) endometritis can also be diagnosed by using urinary test strips (Multistix 10 SG; Bayer Corporation, Elkart, IN, USA) on uterine lavage samples which contains leukocyte esterase, protein, and pH tests, however, in comparison with conventional cytology, 20.6% of cows could not be accurately classified therefore the reagent strip as an alternative test may require further confirmation [20]. It is worth mentioning that besides the difficulties of uterine biopsy in the field it can give low agreement with cytology for the diagnosis of subclinical endometritis [86].

**Treatment of subclinical endometritis**

Subclinical endometritis can be treated with a prostaglandin i.m. injection (cloprostenol 500 mg) or/and an i.u. antibiotic therapy (cephapirin) at 20–33 DIM to improve the reproductive performance [88]. Intrauterine infusion of cefotiofur hydrochloride reduced the prevalence of *T. pyogenes*, but did not affect fertility of dairy cows already receiving PGF$_{2\alpha}$ [77]. One or 2 treatments with PGF$_{2\alpha}$ before initiation of the timed AI program with subclinical endometritis were unable to improve uterine health, pregnancy rate per AI, and maintenance of pregnancy in lactating dairy cows [75]. Intrauterine lavage with 500-600 ml of sterile physiological saline (35°C–40°C) at Day 30 after calving may significantly decrease the number of PMNs in the uterus and improve pregnancy rate however it requires further large scale confirmations [35].

**Prevention of Uterine Diseases**

Cows having hypocalcaemia, dystocia, stillbirth, twins or retained placenta in the periparturient period are more likely to contract uterine infections than those cows that calve normally. Thus, management of sanitation, nutrition, population density, stress to prevent or reduce the incidence of these predisposing factors (especially dystocia) should be impeccable. Therefore prevention remains limited to general guidance on hygiene at calving [89], adequate nutrition (Ca, Se, Vitamin E, etc.) and the control of infectious diseases.

Routine systemic or intra-uterine administration of cefotiofur may be beneficial for the prevention of clinical metritis, however its effect on reproductive performance is not significantly different to that of no treatment therefore it cannot be recommended for the field [6,49,51,90]. Similarly controversial results were reported when a single-dose of cefotiofur crystalline free acid sterile suspension was used in dairy cows at high risk of uterine disease (twin, dystocia, or retained placenta) within 24 hr after calving [28,29].

One of the pharmacological approaches to the prevention and treatment of retained placenta can be the administration of prostaglandin immediately after calving [91], however due to controversy results further studies are needed to confirm its efficacy. Similarly the advantage of treatment with a new Chinese herbal aqua-acupuncture formulation Dang Hong Fu [54] needs further confirmation. Repeated administration of PGF$_{2\alpha}$ to cows on Days 7 and 14 or on Days 22 and 35 after calving had no effect on the prevalence of clinical endometritis at Days 22 and 58 after calving, and there was no effect on the probability of pregnancy after insemination at oestrus among cows with a voluntary waiting period of >100 days, or at timed AI at Day 85 when Presynch was performed [92]. Similarly preventive administration of PGF$_{2\alpha}$ at both 5 and 7 wk after calving had no positive effect on reproduction in dairy cows [28]. Preventive ozone intrauterine (spray) treatment [47,93], Sheng Hua Tang (a classical
herbal formula consisting of Radix Angelicae Sinensis, Ligustici Rhizoma, Semen Persicae, Zingiberis Rhizoma, and Radix Glycyrrhizae [94] or herbal tincture containing Herba Leonuri, Angelicae Sinensis Radix, Flos Carthami, Myrrha and Rhizoma Cyperi [95] during early puerperal period may improve the reproductive efficacy in dairy cows. In contrast, homeopathic drugs like Lachesis composite (Lachesis), Carduus compositum (Carduus), and Traumeel LT (Traumeel) were not effective in preventing bovine endometritis or in enhancing reproductive performance in dairy cows [96].

On the other hand subcutaneous vaccination on Days 230 and 260 of pregnancy with inactivated bacterial components and/or protein subunits of E. coli, F. necrophorum and T. pyogenes may prevent puerperal metritis during the first lactation of dairy cows, leading to improved reproduction however further studies are needed to confirm the benefit of vaccination in the field [97].

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Citation: Szenci O (2016) Recent Possibilities for Diagnosis and Treatment of Post Parturient Uterine Diseases in Dairy Cow. JFIV Reprod Med Genet 4: 170. doi:10.4172/2375-4508.1000170

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