



## Destination of corpus luteum in postpartum clinical endometritis cows and factors affecting self-recovery

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### ABSTRACT

In this study, the fate of corpus luteum was investigated in cows affected by severe clinical endometritis. Also, concentration of IL-1 $\beta$ , IL-6, COX-2, adiponectin, leptin, progesterone (P4), PGFM, insulin, and IGF-1 were studied in severe clinical endometritis cows at day 30  $\pm$  3 postpartum. Eighty-seven dairy cows affected by severe clinical endometritis were selected and their reproductive tract was examined by ultrasonography at day 30  $\pm$  3 postpartum (first examination) and 14 days later (second examination). The majority of the cows with CL and affected by clinical endometritis at first examination had new CL 14 days later, and most of these cows were clean without any treatment (self-healing). The CL of about 28.7% of all cows with clinical endometritis at first examination persisted two weeks later and the uterus of most of them remained infected as pyometric cows. Most of the anestrus cows (83.3%) were pregnant, but only 51% of cows with new CL were pregnant at the end of next six month period. There was a significant difference in the means of lactation number, uterine lumen diameter, cervical diameter, and size of uterine horn, milk production at first examination, concentrations of insulin, COX-2, P4, IL-1 $\beta$ , and IL-6 among persistent CL, new CL, and anestrus cows. The pregnancy rate and the discharge score were different among persistent CL, new CL, and anestrus cows. In conclusion, CL on ovary of cows and its fate could affect recovery from severe clinical endometritis. The concentration of some metabolic hormones influenced the self-recovery of cows from clinical endometritis.

### 1. Introduction

The endometritis is one of the most frequently disorders in postpartum dairy cows and adversely influences reproductive their reproductive performance. Over the years, therapeutic protocols have included supportive care, hormone therapy, and intrauterine antibiotic infusions (Sheldon, Price, Cronin, Gilbert, & Gadsby, 2009). Spontaneous healing occurred in 83.5% of cows affected with endometritis during days 15–61 postpartum (Gautam et al., 2010). In previous studies, the spontaneous recovery rate is reported to be 30–40% of all cows (McDougall, Macaulay, & Compton, 2007; Sheldon et al., 2009). The early postpartum endometritis occurs more frequently in cows with decreased blood neutrophil oxidative activity until the first week of postpartum. Spontaneous healing of endometritis has been observed in cows with increase in blood PMN oxidative burst activity, high intrauterine fluid phagocytic neutrophils, and phagocytic index (Mateus, Lopes da Costa, Bernardo, & Robalo Silva, 2002; Mateus, Lopes da Costa, Carvalho, Serra, & Robalo Silva, 2002). The risk factors for the persistency of clinical endometritis are summer calving, clinically relevant urovagina, early postpartum complications, and clinical

endometritis within 2 months postpartum. Recovery or persistency of endometritis depends on the severity of endometritis rather than the time of diagnosis of endometritis at postpartum period (Gautam et al., 2010).

Factors such as feeding, reproductive management, milk yielding, and post-calving hygiene are known to have an impact on the uterine involution and the number of cows affected by endometritis in particular herds (LeBlanc, 2008). The cytokines and chemokines are secreted by endometrium to regulate uterine inflammatory response to infection (Galvao, Santos, Galvao, & Gilbert, 2011). The immune defense mechanisms are depressed by hormonal status around calving and dairy cows predisposed to the development of uterine infections (Kim, Na, & Yang, 2005). The onset of endometritis has been associated with an increase in the progesterone concentrations (Seals, Matamoros, & Lewis, 2002). The cervix is closed functionally and susceptibility to persistent infection increased in response to the progesterone (Noakes, Wallace, & Smith, 1990). The injection of PGF2 $\alpha$  in cows with CL and affected by clinical endometritis produced better therapeutic effect. PGF2 $\alpha$  induces luteolysis, increases estradiol concentration; then, immune response is likely to up-regulate and uterus would be able

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to overcome infection (Lewis, 1997). It can be assumed that there is a relationship between the retention of CL, probably the presence of risk factors such as metabolic hormones, reproductive hormones, cytokines, and reproductive parameters and persistence of endometritis.

In most of the cows with a normal postpartum period, first shortened lifespan of the CL is developed following the ovulation of dominant follicle by day 30 after calving (Roche, Crowe, & Boland, 1992). The early release of PGF2 $\alpha$  is observed when uterus was not exposed to P4 before the first post-calving estrus and ovulation. This phenomenon occurs due to decreased P4 receptors and up regulation of oxytocin receptors (Cooper, Carver, Villeneuve, Silvia, & Inskeep, 1991). Length of the next estrous cycle is significantly longer and maximum concentration of progesterone is higher than the early post-calving estrous cycle. A strong relationship was observed between maximum diameter of corpus luteum and peak of plasma P4 level in the second estrous cycle (Rajamahendran, & Taylor, 1990). Ovarian follicle growth and function is affected adversely by uterine infections and leads to the formation of smaller and less steroidogenic follicles (Sheldon, Noakes, Rycroft, Pfeiffer, & Dobson, 2002).

Loss of body condition in dairy cows during periparturient period is related to several post-calving uterine infections (Kasimanickam et al., 2013). Cytokines and/or cytokine-mediated neural and endocrine hormones are the main factors associated with a rapid and intensive loss of body weight (Kasimanickam et al., 2013). Length and intensity of the early postpartum negative energy balance (NEB) are associated with the time of restoration of ovarian activity after calving (S. T. Butler, Pelton, & Butler, 2006).

It is well known that blood glucose, fatty acids, amino acids, insulin, leptin, and adiponectin directly regulate fertility at level of the hypothalamus-pituitary-gonadal axis (Tabandeh, Hosseini, Saeb, Kafi, & Saeb, 2010). The low GnRH and LH pulse frequency may not be affected by low blood insulin and IGF-I, but high level of insulin and IGF-I could stimulate ovarian estradiol production (Butler, Pelton, & Butler, 2004). Production of cytokine, monocytes/macrophages activity, angiogenesis, wound healing, and hematopoiesis may be affected by leptin (Kasimanickam et al., 2013). Adiponectin controls metabolism of lipid, insulin sensitivity, and glucose homeostasis (Lemor, Hosseini, Sauerwein, & Mielenz, 2009; Maillard et al., 2010; Moschen et al., 2007).

In our previous study, it was confirmed that PGF2 $\alpha$  was a more effective treatment in cows with CL on their ovary and affected by clinical endometritis (Ahmadi, Mogheiseh, Mirzaei, Nazifi, & Fallah, 2018). Also, it was indicated that some dominant follicles ovulate in cows with infected uterus and corpus luteum may persist for a long time (Sheldon, Williams, Miller, Nash, & Herath, 2008). This experiment was aimed to study the destination of corpus luteum in cows with CL on their ovary, affected by severe clinical endometritis and that did not receive any treatment at day 30  $\pm$  3 postpartum. Changes in ovarian structures, IL-1 $\beta$ , IL-6, COX-2, adiponectin, leptin, progesterone (P4), PGFM, insulin, and IGF-1 were monitored in severe clinical endometritis cows that improved spontaneously after first examination. Also, cell count at first milk sampling, production at first milk sampling, production at second milk sampling, cell count at second milk sampling, AI number, BCS, and lactation number were studied in association with the recovery from severe clinical endometritis in postpartum dairy cows. We considered possible risk factors associated with spontaneous lysis of corpus luteum and persistent corpus luteum in dairy cows affected by severe clinical endometritis.

## 2. Material and methods

### 2.1. Statement of animal rights

The study was conducted under the confirmation of the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). Moreover, the protocols of European Council Directive

(2010/63/EU) of September 22, 2010, about the safekeeping of animals considered for experimental studies.

### 2.2. Animals and herds

The study was conducted in a commercial Holstein dairy farm ( $n = 2500$ ) near Shiraz, Fars province, in the central part of Iran (29°58'34"N, 52°40'45"E). The cows were enrolled from October 2015 to August 2016. Artificial insemination was used exclusively after a voluntary waiting period of approximately 50 days and all cows were milked three times daily, with approximately 8 h intervals, and average milk production was about 52 kg/day. Cows were calved in the clean calving open shed house. Additionally, cows were fed thrice daily with a standard formulation to meet the nutritional needs for dairy cows. These cows did not receive any medical treatment at least 14 days before sampling.

### 2.3. Clinical examinations

Postpartum palpation of the reproductive tract and the ultrasonography examination of ovaries were conducted with 5 MHz rectal linear probe (Easyscan<sup>®</sup>, BCF, UK) and for the detection of corpus luteum on day 30  $\pm$  3 (first examination). The cows were examined for the observation of fresh discharge around the vulva, perineum, or tail. The manual vaginal examination was performed if a discharge was not visible externally (Sheldon, Lewis, LeBlanc, & Gilbert, 2006; Yavari, Haghkhah, Ahmadi, Gheisari, & Nazifi, 2009). The severe clinical endometritis was characterized with purulent or mucopurulent (>50% pus) discharges, cervical diameter >7.5 cm, palpable uterine lumen, and lumen filled with pus in ultrasound examination (LeBlanc et al., 2002). Blood samples were collected from the coccygeal vein into tubes and transported on ice to the laboratory. Serum was separated by blood centrifugation at 750  $\times$  g for 10 min and stored at  $-20^{\circ}\text{C}$ . The selected cows were evaluated 14 days later (second examination) to determine their uterine condition (clean: cows cured from severe clinical endometritis and there was not any clinical, rectal palpation and ultrasound signs. dirty: cows did not improve from severe clinical endometritis and there was clinical, rectal palpation or ultrasound signs. pyometra: cows considered pyometric that there was a CL on their ovary and endometritis was not improved spontaneously), ovarian structures (CL and follicles), and the destination of CL that had been observed in the first examination (regressed and developed new CL, persistent CL and anestrus). Cows were diagnosed anestrus when there were no active structures on their ovary at first and second examinations. The development of new CL was confirmed based on presence of new CL on the ipsilateral or contra lateral ovary, heat detection by skilled person and verification of estrus in ultrasound examination. After the second examination, the dirty cows were treated according to the common treatment protocols with oxytetracycline 10% and PGF2 $\alpha$  of the dairy farm (Ahmadi et al., 2018).

Also, at first examination the cows were divided into three groups according to the size and the position of reproductive system (SPS): cows were diagnosed as having small (SPS1), medium (SPS2), or large reproductive tracts (SPS3). Cows considered SPS1 had small and involuted uterine horns that remained within the pelvic cavity; the reproductive tracts of cows with SPS2 were intermediate in uterine horn and cervical diameter and longer uterine horns leaving partial pelvic cavity; and SPS3 cows had reproductive tracts that were larger and rested mostly outside the pelvic cavity (Young et al., 2017).

Data related to milk production and somatic cell count were collected at the time of first examination (first milk sampling) and second examination (second milk sampling). Self-recovery of cows from severe clinical endometritis and cows treated with common protocols of farm artificially inseminated at the first estrus following treatment based on AM/PM role after standing heat were detected. The cows were examined with transrectal ultrasonography for pregnancy diagnosis 30

days after artificial insemination and data related to reproductive and production performance were collected during the next 6 months.

#### 2.4. Laboratory methods and measurements

Serum levels of IL-1 $\beta$  (sensitivity 15.6 pg/ml; intra-assay precision CV < 8%; inter-assay precision CV < 10%), IL-6 (sensitivity 2.5 pg/ml; intra-assay precision CV < 15%; inter-assay precision CV < 15%), leptin (sensitivity 3.12 ng/ml; intra-assay precision CV < 15%; inter-assay precision CV < 15%), and insulin (sensitivity 32.28 pmol/ml; intra-assay precision CV < 10%; inter-assay precision CV < 12%) were measured by a quantitative sandwich enzyme immunoassay using commercial bovine-specific kits (Shanghai Crystal Day Biotech Co., Ltd. China). Serum levels of adiponectin (sensitivity 3.125  $\mu$ g/ml; intra-assay precision CV < 15%; inter-assay precision CV < 15%), progesterone (sensitivity 0.2 ng/ml; intra-assay precision CV < 15%; inter-assay precision CV < 15%), PGFM (sensitivity 20.8 pg/ml; intra-assay precision CV < 10%; inter-assay precision CV < 10%), and IGF-1 (sensitivity 0.94 ng/ml; intra-assay precision CV < 15%; inter-assay precision CV < 15%) were measured by a quantitative sandwich enzyme immunoassay using commercial bovine-specific kits (Cusabio Biotech Co., Ltd, Wuhan, Hubei, China). Serum levels of COX-2 (sensitivity 1.2 ng/ml; intra-assay precision CV < 10%; inter-assay precision CV < 10%) were measured by a quantitative sandwich enzyme immunoassay using commercial bovine-specific kits (Lifespan, Biosciences, Inc. America).

#### 2.5. Statistical analysis

The results were analyzed with SPSS (Version 16.0, SPSS Inc., Chicago, Illinois). The variables in terms of the persistent CL, new CL and anestrus, infected (dirty) and/or non-infected (clean), and pregnant and/or non-pregnant cows were considered in the period of self-recovery for the affected cows with severe clinical endometritis. The chi-square and normality test were applied for studying the variables and reproductive parameters during the self-cure. There was a normal distribution, so ANOVA or Student's *t*-test was applied. The average value of the variables (IL-1 $\beta$ , IL-6, COX-2, adiponectin, leptin, progesterone (P4), PGFM, insulin and IGF-1) and the reproductive and productive parameters (AI number, somatic cell count at first milk sampling, production at first milk sampling, production at second milk sampling, somatic cell count at second milk sampling, BCS and lactation) were evaluated in relation to the self-recovery of cows from severe clinical endometritis. *P* values  $\leq$  0.05 was considered significant.

### 3. Results

One hundred and twenty-three cows were affected by severe clinical endometritis from 1150 cows that were examined. Some cows were removed from study because of unexpected diseases or their ovulation could not be confirmed. So, eighty-seven severe clinical endometritis cows remained. At the first rectal ultrasonography (first examination), corpus luteum was detected on the ovary of 74 cows and 13 cows were anestrus.

At first examination, there was CL on ovary of 74/87 cows affected by severe clinical endometritis. Two weeks after first examination, the cows were monitored for the destination of corpus luteum that was detected at first examination. New CL was detected in 49 cows from 74 cows that had CL at the first examination; CL was persistent in 25 cows with CL at the first examination. The uterus of most cows with new CL was clean compared to cows with persistent CL (67.3 vs. 28%, *P* < 0.05). Most of the anestrus cows were pregnant in comparison to cows with new CL (83.3 vs. 51%, *P* < 0.05) at the end of the study (Fig. 1).

The results of comparison of mean ( $\pm$  SD) concentration of metabolites and hormones (at first examination, 30  $\pm$  3 post partum) and

reproductive and productive factors in cows with new CL (*n* = 49), persistent CL (*n* = 25) or anestrus (*n* = 13) were presented in Table 1. Comparing mean concentration of progesterone (P4), insulin, COX-2, IL-1 $\beta$ , IL-6, lactation number, first milk sample production, uterine lumen diameter, cervical diameter, and the diameter of uterine horn showed significant differences between persistent CL, new CL, and anestrus cows. There was significant lower percentage of the clean uterus in persistent CL (28%) compared with new CL groups (67.3%). In addition, there was significant lower pregnancy rate in new CL (51%) compared with anestrus (83.3%) groups (Table 2).

In Table 3, cows were aligned into three groups based on uterine location, including pelvic cavity (SPS1), retractable (SPS2), and over pelvic brim (SPS3). Results indicated that frequency of clean uterus in SPS2 group (56.9%) was significantly higher than that of SPS3 group (22.2%), but the pregnancy rate was not significantly different between these groups.

In Table 4, 67.34% of cows with a new CL were recovered from severe clinical endometritis (clean cows). The results showed that levels of insulin, adiponectin, IL-1 $\beta$ , uterine horn diameter, and days open were significantly different between clean and dirty new CL cows.

The same comparisons were considered for the persistent CL cows in Table 5. Results indicated that 28% of cows with persistent CL recovered from clinical endometritis (clean cows) and 72% of them remained infectious and progressed to pyometra. The mean concentration of IL-6, IL-1 $\beta$ , first milk sample cell count, uterine lumen diameter, and uterine horn diameter were significantly different between clean and infected cows with persistent CL.

Another comparison was performed between clean and dirty cows that were in anestrus two weeks after first examination (Table 6). About 50% of these cows remained infected. The average concentration of insulin and IL-6, first milk sample cell count, and days open were significantly different between clean and dirty anestrus cows.

### 4. Discussion

Most of the cows with CL and affected by severe clinical endometritis at first examination (30  $\pm$  3 days postpartum) had new CL 14 days later (at second examination) and most of them were clean without any treatment. The CL of about 28.7% of all cows with severe clinical endometritis at first examination persisted two weeks later and uterus of most of them remained infected (72%) as pyometric cows. Concentration of some metabolic or steroid hormones and productive and reproductive factors affected the self-recovery from severe clinical endometritis in cows with new CL, persistent CL or anestrus cows. Also, size and position score of the reproductive tract affected self-healing of uterus from severe clinical endometritis.

Results indicated that there was no significant difference in level of progesterone (P4) between persistent CL and new CL cows in 30  $\pm$  3 days postpartum. This result may explain with the findings reported by Williams et al. (2007) and Strüve et al. (2013). They demonstrated that first dominant follicle of post-calving was smaller and secreted lower estradiol in infected uterine cows compared with normal cows. In addition, they found that infected cows had smaller CLs on their ovary and produced less plasma progesterone (P4) than normal cows (Strüve et al., 2013; Williams et al., 2007). However, P4 level in anestrus cows was significantly lower than other cows because there was not any luteal structure on ovary (Strüve et al., 2013; Williams et al., 2007).

The significant difference in percentage of clean uterus was observed between persistent CL and new CL cows (Table 2). It confirms the findings of Mateus, Lopes da Costa, Bernardo et al. (2002) and Mateus, Lopes da Costa, Carvalho et al. (2002) that the resumption of post-calving ovarian activity might have been affected by the endotoxin absorbance from the infected uterus into the blood in cows affected by severe endometritis (Mateus, Lopes da Costa, Bernardo et al., 2002, Mateus, Lopes da Costa, Carvalho et al. 2002). Indirectly, it may affect and delay uterine clearance and elimination of contamination.

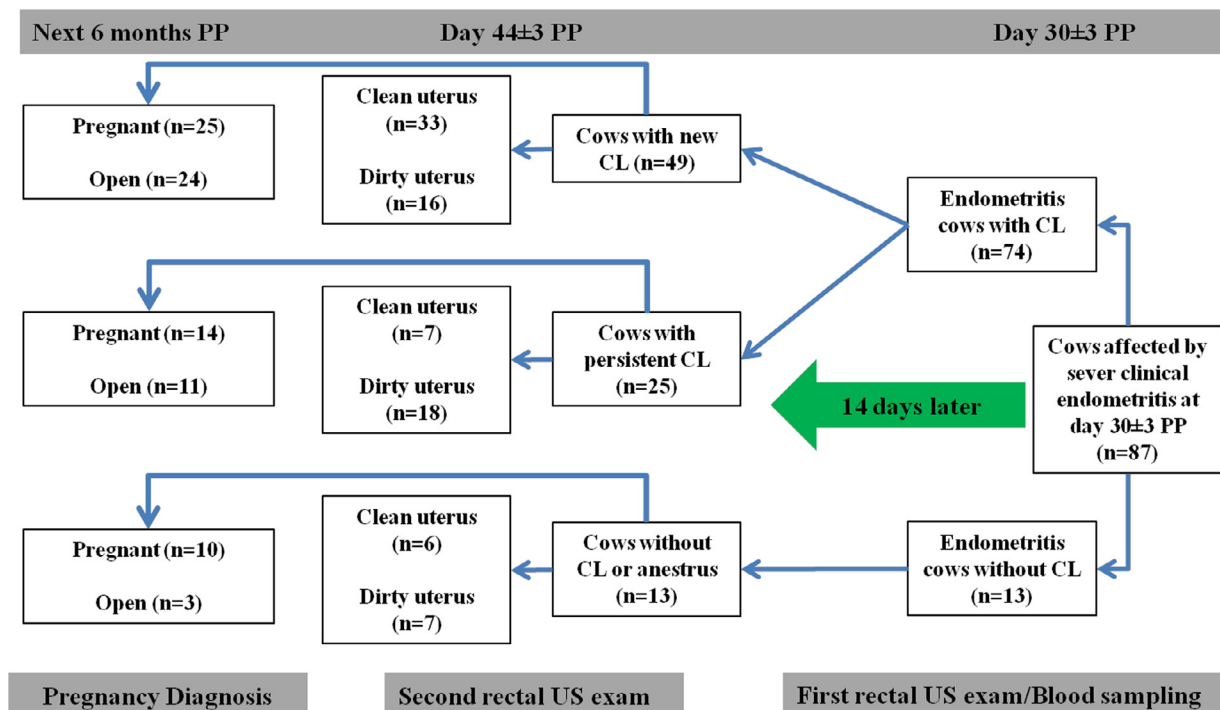


Fig. 1. This diagram presented dairy cows affected by severe clinical endometritis with or without CL on their ovary at day 30 ± 3 postpartum (PP). Some cows were improved during 14 days, spontaneously. Pregnancy rate in anestrus cows occurred following common treatment protocols in farm. Ultrasonography (US).

Table 1

Comparison of mean ( ± SD) concentration of metabolic, hormones (at clean test, 30 ± 3) and reproductive and productive factors in cows (n = 87) with persistent CL, new CL or anestrus cows.

Variables	New CL (n = 49)	Persistent CL (n = 25)	Anestrus (n = 13)
P4 (ng/ml)	2.58 ± 1.86 <sup>a</sup>	2.44 ± 1.56 <sup>a</sup>	0.63 ± 0.83 <sup>b</sup>
IGF-1 (ng/L)	34.00 ± 5.63	34.61 ± 6.14	35.26 ± 5.57
PGFM (pg/L)	2.73 ± 0.97	2.61 ± 1.16	2.66 ± 1.13
COX-2 (ng/ml)	112.36 ± 61.79 <sup>a</sup>	101.79 ± 42.58 <sup>a</sup>	152.03 ± 57.91 <sup>b</sup>
Leptin (ng/ml)	3.90 ± 0.12	3.92 ± 0.12	3.93 ± 0.14
Adiponectin (µg/ml)	426.82 ± 12.76	423.38 ± 13.32	419.73 ± 11.27
Insulin (pMol/ml)	39.41 ± 8.76 <sup>a</sup>	35.22 ± 8.19 <sup>b</sup>	32.09 ± 7.02 <sup>b</sup>
IL-1β (pg/ml)	1.73 ± 0.2 <sup>a</sup>	1.64 ± 0.19	1.56 ± 0.18 <sup>b</sup>
IL-6 (pg/ml)	106.71 ± 29.82 <sup>a</sup>	86.10 ± 18.49 <sup>b</sup>	90.72 ± 21.15 <sup>b</sup>
Lactation number	2.74 ± 1.32 <sup>a</sup>	3.24 ± 1.09 <sup>a</sup>	1.61 ± 1.26 <sup>b</sup>
First milk sample production (kg)	46.07 ± 11.11 <sup>a</sup>	39.36 ± 14.33 <sup>b</sup>	40.75 ± 7.12
First milk sample cell count (cell/ml)	123.89 ± 375.39	113.13 ± 280.49	105.66 ± 269.56
Second milk sample production (kg)	45.53 ± 9.74	40.86 ± 15.19	40.66 ± 7.31
Second milk sample cell count (cell/ml)	64.81 ± 195.94	63.68 ± 139.58	37.75 ± 66.40
BCS	2.89 ± 0.28	2.90 ± 0.31	2.89 ± 0.16
Diameter of uterine lumen (cm)	1.65 ± 1.05	2.04 ± 1.17 <sup>a</sup>	1.15 ± 0.68 <sup>b</sup>
Cervical diameter (cm)	4.86 ± 1.32 <sup>a</sup>	5.62 ± 1.86 <sup>b</sup>	5.19 ± 1.85
Diameter of (larger) uterine horn (cm)	7.08 ± 4.22	7.96 ± 4.84 <sup>a</sup>	4.69 ± 1.68 <sup>b</sup>
Thickness of uterine wall (cm)	1.69 ± 0.54	1.80 ± 0.64	1.46 ± 0.51
AI number	1.93 ± 1.08	1.95 ± 0.89	1.50 ± 0.79
DO (day)	113.34 ± 39.80	110.54 ± 36.08	105.75 ± 20.35

<sup>a,b</sup>Different superscript letters indicate a significant difference in the same rows ( $P \leq 0.05$ ).

Table 2

Comparing the frequency of clean and pregnant cows with persistent CL, new CL and anestrus cows.

Variables	New CL	Persistent CL	Anestrus
Clean	67.3% (33/49) <sup>a</sup>	28.0% (7/25) <sup>b</sup>	46.2% (6/13)
Pregnant	51.0% (25/49) <sup>a</sup>	63.6% (14/25)	83.3% (10/13) <sup>b</sup>

<sup>a,b</sup>Different superscript letters indicate a significant difference in the same rows ( $P \leq 0.05$ ).

Table 3

Comparing the frequency of cleaning and pregnancy condition in cows that their uterine location was in pelvic cavity, retractable or over pelvic brim.

Variables	In the pelvic cavity (SPS1)	Retractable (SPS2)	Over pelvic brim (SPS3)
Clean	53.8% (7/13)	56.9% (37/65) <sup>a</sup>	22.2% (2/9) <sup>b</sup>
Pregnant	61.5% (8/13) <sup>a</sup>	61.9% (39/65)	37.5% (3/9) <sup>b</sup>

<sup>a,b</sup>Different superscript letters indicate a significant difference in the same rows ( $P \leq 0.05$ ).

**Table 4**

Comparison of mean ( $\pm$  SD) concentration of metabolic hormones and reproductive and productive factors in new CL cows ( $n = 49$ ) that were cleaned or infectious at two weeks after clean test time.

Variables	Clean ( $n = 33$ )	Infectious ( $n = 16$ )	P-value
P4 (ng/ml)	2.26 $\pm$ 1.77	3.23 $\pm$ 1.91	0.03
IGF-1 (ng/L)	34.17 $\pm$ 6.07	33.66 $\pm$ 4.77	0.80
PGFM (pg/L)	2.86 $\pm$ 0.98	2.46 $\pm$ 0.90	0.20
COX <sub>2</sub> (ng/ml)	116.99 $\pm$ 65.20	102.81 $\pm$ 54.82	0.50
Leptin (ng/ml)	3.89 $\pm$ 0.12	3.92 $\pm$ 0.11	0.40
Adiponectin ( $\mu$ g/ml)	424.30 $\pm$ 11.85 <sup>a</sup>	432.01 $\pm$ 13.38 <sup>b</sup>	0.04
Insulin (pMol/ml)	41.26 $\pm$ 7.91 <sup>a</sup>	35.59 $\pm$ 9.44 <sup>b</sup>	0.03
IL-1 $\beta$ (pg/ml)	1.77 $\pm$ 0.2 <sup>a</sup>	1.65 $\pm$ 0.18 <sup>b</sup>	0.05
IL-6 (pg/ml)	112.28 $\pm$ 29.27	95.22 $\pm$ 28.45	0.06
Lactation number	2.57 $\pm$ 1.34	3.14 $\pm$ 1.23	0.10
First milk sample production (kg)	45.60 $\pm$ 11.27	47.03 $\pm$ 11.09	0.60
First milk sample cell count (cell/ml)	78.69 $\pm$ 160.74	199.72 $\pm$ 582.98	0.08
Second milk sample production (kg)	44.78 $\pm$ 8.86	47.06 $\pm$ 11.49	0.60
Second milk sample cell count (cell/ml)	30.21 $\pm$ 37.64	123.22 $\pm$ 316.29	0.09
BCS	2.85 $\pm$ 0.26	2.96 $\pm$ 0.31	0.30
Diameter of uterine lumen (cm)	1.48 $\pm$ 1.03	2.00 $\pm$ 1.03	0.10
Cervical diameter (cm)	4.80 $\pm$ 1.36	5.00 $\pm$ 1.26	0.60
Diameter of (larger) uterine horn (cm)	5.90 $\pm$ 2.77 <sup>a</sup>	9.83 $\pm$ 5.93 <sup>b</sup>	0.04
Thickness of uterine wall (cm)	1.66 $\pm$ 0.59	1.75 $\pm$ 0.44	0.50
AI number	1.96 $\pm$ 0.98	1.87 $\pm$ 1.31	0.60
DO (day)	104.93 $\pm$ 38.29 <sup>a</sup>	130.68 $\pm$ 38.28 <sup>b</sup>	0.05

<sup>a,b</sup>Values with different indices differ significantly ( $P \leq 0.05$ ).

**Table 5**

Comparison of mean ( $\pm$  SD) concentration of metabolic hormones and reproductive and productive factors in persistent CL cows ( $n = 25$ ) that were cleaned or infectious at two weeks after clean test time.

Variables	Clean ( $n = 7$ )	Infectious ( $n = 18$ )	P-value
P4 (ng/ml)	2.55 $\pm$ 1.39	2.40 $\pm$ 1.66	0.40
IGF-1 (ng/L)	37.00 $\pm$ 7.16	33.68 $\pm$ 5.65	0.20
PGFM (pg/L)	2.35 $\pm$ 0.91	2.71 $\pm$ 1.26	0.40
COX <sub>2</sub> (ng/ml)	121.24 $\pm$ 28.34	94.22 $\pm$ 45.38	0.10
Leptin (ng/ml)	3.89 $\pm$ 0.13	3.93 $\pm$ 0.11	0.40
Adiponectin ( $\mu$ g/ml)	426.48 $\pm$ 16.85	422.18 $\pm$ 12.03	0.40
Insulin (pMol/ml)	37.96 $\pm$ 7.18	33.93 $\pm$ 7.87	0.26
IL-1 $\beta$ (pg/ml)	1.76 $\pm$ 0.11 <sup>a</sup>	1.59 $\pm$ 0.20 <sup>b</sup>	0.05
IL-6 (pg/ml)	96.82 $\pm$ 11.31 <sup>a</sup>	81.93 $\pm$ 19.28 <sup>b</sup>	0.04
Lactation number	3.28 $\pm$ 1.11	3.22 $\pm$ 1.11	0.80
First milk sample production (kg)	42.66 $\pm$ 14.17	38.12 $\pm$ 14.64	0.40
First milk sample cell count (cell/ml)	10 $\pm$ 24.49 <sup>a</sup>	184.65 $\pm$ 344.08 <sup>b</sup>	0.03
Second milk sample production (kg)	45.00 $\pm$ 17.05	39.31 $\pm$ 14.73	0.30
Second milk sample cell count (cell/ml)	45 $\pm$ 17.05	38.57 $\pm$ 15.66	0.42
BCS	2.78 $\pm$ 0.30	2.94 $\pm$ 0.31	0.30
Diameter of uterine lumen (cm)	1.14 $\pm$ 1.06 <sup>a</sup>	2.38 $\pm$ 1.03 <sup>b</sup>	0.02
Cervical diameter (cm)	5.00 $\pm$ 1.41	5.86 $\pm$ 1.99	0.30
Diameter of (larger) uterine horn (cm)	5.57 $\pm$ 1.51 <sup>a</sup>	8.88 $\pm$ 5.38 <sup>b</sup>	0.02
Thickness of uterine wall (cm)	1.57 $\pm$ 0.53	1.88 $\pm$ 0.67	0.20
AI number	2.00 $\pm$ 1.26	1.93 $\pm$ 0.77	0.80
DO (day)	108.16 $\pm$ 42.99	111.43 $\pm$ 34.69	0.80

<sup>a,b</sup>Values with different indices differ significantly ( $P \leq 0.05$ ).

**Table 6**

Comparison of mean ( $\pm$  SD) concentration of metabolic hormones and reproductive and productive factors in anestrus cows ( $n = 13$ ) that were cleaned or infectious at two weeks after clean test time.

Variables	Clean ( $n = 6$ )	Infectious ( $n = 7$ )	P-value
P4 (ng/ml)	1.07 $\pm$ 1.10	0.25 $\pm$ 0.17	0.10
IGF-1 (ng/L)	33.88 $\pm$ 4.18	36.43 $\pm$ 6.62	0.40
PGFM (pg/L)	2.35 $\pm$ 1.45	2.92 $\pm$ 0.79	0.30
COX <sub>2</sub> (ng/ml)	139.94 $\pm$ 37.17	162.39 $\pm$ 72.70	0.40
Leptin (ng/ml)	3.93 $\pm$ 0.15	3.93 $\pm$ 0.14	0.90
Adiponectin ( $\mu$ g/ml)	420.17 $\pm$ 12.27	419.36 $\pm$ 11.31	0.90
Insulin (pMol/ml)	37.18 $\pm$ 6.02 <sup>a</sup>	27.73 $\pm$ 4.52 <sup>b</sup>	0.008
IL-1 $\beta$ (pg/ml)	1.65 $\pm$ 0.18	1.47 $\pm$ 0.14	0.07
IL-6 (pg/ml)	105.95 $\pm$ 19.94 <sup>a</sup>	77.66 $\pm$ 11.54 <sup>b</sup>	0.009
Lactation number	2.00 $\pm$ 1.67	1.28 $\pm$ 0.75	0.30
First milk sample production (kg)	38.5 $\pm$ 6.71	43.00 $\pm$ 7.37	0.20
First milk sample cell count (cell/ml)	207.5 $\pm$ 367.34 <sup>a</sup>	3.83 $\pm$ 6.14 <sup>b</sup>	0.04
Second milk sample production (kg)	41.83 $\pm$ 7.35	39.50 $\pm$ 7.76	0.90
Second milk sample cell count (cell/ml)	24.16 $\pm$ 18.57	51.33 $\pm$ 94.4	0.51
BCS	2.95 $\pm$ 0.11	2.85 $\pm$ 0.16	0.30
Diameter of uterine lumen (cm)	1.33 $\pm$ 1.03	1.00 $\pm$ 00	0.40
Cervical diameter (cm)	5.16 $\pm$ 2.46	5.21 $\pm$ 1.34	0.50
Diameter of (larger) uterine horn (cm)	5.25 $\pm$ 2.40	4.21 $\pm$ 0.56	0.20
Thickness of uterine wall (cm)	1.33 $\pm$ 0.51	1.57 $\pm$ 0.53	0.50
AI number	1.50 $\pm$ 0.83	1.50 $\pm$ 0.83	1.00
DO (day)	95.33 $\pm$ 21.76 <sup>a</sup>	116.16 $\pm$ 13.31 <sup>b</sup>	0.04

<sup>a,b</sup>Values with different indices differ significantly ( $P \leq 0.05$ ).

The significant difference in the pregnancy rate that was observed in this study between new CL and anestrus cows (Table 2) was in accordance with the findings of previous studies. They emphasized that there was a significant relationship between incidence of persistent CLs and disturbed uterine involution in dairy cows (Strüve et al., 2013; Taylor, Beever, Bryant, & Wathes, 2003). In our study, because more anestrus cows remained infected, they were treated with common treatment protocol. As a result, it can be concluded that the reason for the significant increase of pregnant cows in the anestrus group compared to other groups was performing the common treatment protocols of farm.

In the present study, mean concentration of IGF-1 was not different among persistent CL, new CL, and anestrus cows and between clean and dirty uterine cows. Butler et al. (2006) reported that concentration of IGF-1 decreased in plasma of infected cows (Butler et al., 2006). In addition, they demonstrated the reduction of circulating concentrations of IGF-1 was associated with an impaired reproductive performance (Butler et al., 2006). Their results were different from the results presented in this study. It may be because we did not compare cows with infected uterus with normal cows. All cows in our study were affected by severe clinical endometritis at the first examination.

A significant higher level of adiponectin was observed in dirty uterine cows than clean cows with new CL. Adiponectin possesses anti-inflammatory effect and could influence the reproductive system by affecting the hypothalamus-pituitary axis (Kubota et al., 2007; Rodriguez-Pacheco et al., 2007; Wen et al., 2008). Body loss increases adiponectin in cows with uterine infections (Kasimanickam et al., 2013). However, significant changes of adiponectin were not observed in persistent CL or anestrus cows whose uterus was clean or dirty. Physiological level of adiponectin could increase progesterone and estradiol secretions in response to adding IGF-I in cultured granulosa cells in rat, chicken and human (Maillard et al., 2010).

The mean insulin concentration was significantly different among persistent CL, new CL, and anestrus cows. In addition, the

concentration of insulin was different in new CL and anestrus cows whose uterus was clean or dirty. The concentration of insulin could affect the ovulation rate and uterine cleaning following severe clinical endometritis. Others indicated that reduced circulating insulin may not be related with low GnRH and LH pulse frequency, but the production of ovarian estradiol may be stimulated by high levels of insulin (Butler et al., 2004). In the present study, the level of insulin concentration was higher in clean cows than dirty cows with new CL and anestrus. This finding is in agreement with other studies that reported insulin could stimulate the overall metabolism, mitosis of cultured bovine granulosa cells, and glucose uptake (Langhout, Spicer, & Geisert, 1991). It can be assumed that insulin can stimulate defense mechanism through promoting ovarian function. On the other hand, an increased energy intake can affect the body's immune system. Postpartum ovarian rebound is interdependent on the elimination of uterine bacterial contamination by innate immune system, rapid uterine involution, and a short period to the negative energy balance nadir (Silva et al., 2008).

The concentration of IL-6 was significantly different between persistent CL, new CL, and anestrus cows, but IL-1 $\beta$  levels were different only between new CL and anestrus cows and between new CL cows whose uterus was clean or infected. In persistent CL cows, the levels of IL-6 and IL-1 $\beta$  were significantly different between clean and dirty cows, but in anestrus cows, only the level of IL-6 was significantly different between clean and dirty cows. Other researchers indicated that IL-1 $\beta$  is an important mediator of inflammation (Narko, Ritvos, & Ristimäki, 1997) and IL-6 could play an important role in the defense system (Shuster, Kehrl, & Stevens, 1993) and/or could act as a local (paracrine or autocrine) regulator of ovarian function (Adashi, 1990). Therefore, higher levels of these mediators in new CL cows or clean cows at the time of first examination could indicate good prognosis for overcoming uterine infection and normal function of ovaries in postpartum cows.

The concentration of COX-2 was significantly higher in anestrus cows than other cows at first examination. It may be related to severity of the inflammatory response in different cows at first examination that affects the ovarian function. Researchers reported that COX-2 gene transcription in blood of cows with normal puerperium were the same as that found in cows affected by metritis/endometritis. Therefore, it is not a helpful biomarker for determining attendance of puerperal uterine infections (Silva et al., 2008).

Other parameters such as mean lactation number, cervical diameter, diameter of uterine lumen, diameter of uterine horn, and production at first milk sampling were significantly different between cows with persistent CL, new CL, and anestrus. As Lewis (1997) pointed out the causes that affect uterine involution are important because they can lead to subfertility in the future (Lewis, 1997). Mean diameter of the uterine horn in clean cows was significantly lower than that of dirty ones in new CL group. In new CL and anestrus cows, mean number of days open in clean cows was significantly lower than that of dirty ones. In the persistent CL cows, diameter of uterine lumen, diameter of uterine horn, and first milk sample cell count of clean cows were significantly lower than those detected for dirty ones. Uterine involution involves the regeneration of the endometrium, sloughing of the caruncles, necrosis, physical shrinkage, and uterine contractions (Shuster et al., 1993), and severe clinical endometritis delayed physical involution of the uterus (Mateus, Lopes da Costa, Bernardo et al., 2002, Mateus, Lopes da Costa, Carvalho et al. 2002). It could be the reason for the smaller diameter of uterine horn that was observed in the clean cows. Low number of open days in clean cows can be justified by the findings of other researchers who stated that the reproductive efficiency was related to the health condition of cows in the weeks of just before and after calving; also, timely establishment of next pregnancy (De Vries, 2006). Abnormal ovarian activity (prolonged anestrus, prolonged luteal phase and ovarian cysts) more reported in cows with severe endometritis than normal cows (L Mateus, Lopes da Costa, Bernardo et al., 2002, Mateus, Lopes da Costa, Carvalho et al. 2002).

While in cows with persistent CL, there was no difference in the number of open days between groups of cleaned and remained infected cows. In the anestrus group, the cell count at first milk sampling of infectious cows was significantly lower than that in clean cows. This may be explained by the fact that if ovaries are not active, the effect of cell count at first milk sampling may not be observed in the recovery process. Also, pregnancy will occur in with delay (longer open days) in cows with abnormal ovarian activity.

The most cows with size and position score of reproductive tract 1 and 2 were clean and pregnant in compare with cows with SPS3 score. Higher rate of pregnancy per artificial insemination was reported for cows were SPS1 than cows were SPS2 or SPS3 (Young et al., 2017). Diameter, volume and length of uterus affect pregnancy rate in cows. Pregnancy rate is higher in cows with smaller uterus (Baez, Barletta, Guenther, Gaska, & Wiltbank, 2016).

Finally, it was revealed that the corpus luteum of about 33.78% of cows with CL and affected by severe clinical endometritis was not lysed within two weeks and remained persistent. Also, uterus of about 72% of the persistent CL cows remained infected and progressed towards pyometra. Therefore, cows with CL and affected by severe clinical endometritis should receive PGF2 $\alpha$  at clean test. About 50% of uterus of anestrus cows remained infected; thus, they should receive one of the alternative treatments that were proposed in the previous study (Sheldon et al., 2006).

## 5. Conclusions

In conclusion, in cows affected by severe clinical endometritis, concentration of adiponectin, insulin, IL-1 $\beta$ , and IL-6 at clean test could affect ovulation and frequency of clean or dirty uterus two weeks later. Ovulation and size and location of uterus in cows affected by severe clinical endometritis could affect the pregnancy rate and frequency of clean or dirty uterus. Parameters related to uterine involution were significantly different between new CL and anestrus, clean and dirty cows. Subsequently, the reproductive performance indices such as days open were significantly lower in new CL and anestrus cows whose uterus was cleaned.

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## Ethical statement

The study was conducted under the confirmation of the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). Moreover, the protocols of European Council Directive (2010/63/EU) of September 22, 2010, about the safekeeping of animals considered for experimental studies.

## Data for reference

Data are available on <https://figshare.com/s/190c11a96ead9e5084bb>.

## Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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