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DISTRIBUTION AND SIZE OF CORPORA LUTEA IN DAIRY COWS DURING PUERPERIUM

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Abstract: During puerperium phase in cows, uterus goes through involution process, while ovaries restore supressed cyclicity as a result of gestation. After 10-20 days postpartum (PP) luteinizing hormone (LH) levels begin to raise and renewal of cyclicity after parturition is probably most important factor for cows to successfuly conceive again. Almost 95% of dairy cows should restore ovarian cyclicity up to 50 days postpartum. LH surge is important for ovulation and luteinisation of granulosa and theca cells into luteal cells and proliferation of blood vessels. Up to 79% of newly formed *corpora lutea* have central vacuola, filled with fluid. The study involved the total of 54 Holstein-Friesian cows, during first 40 days of lactation. Examinations of the ovaries, were performed in the period from 10 to 40 days postpartum. Ovaries and *corpora lutea* were first palpated and then examined using portable diagnostic ultrasound linear scanner MyLab®30 VETGold portable ultrasound linear scanner with endorectal linear probe LV 513, 5-7.5 MHz (both Esaote SpA, Italy). The same equipment was used to monitor BCS, while lamenes was assessed using Zinpro Locomotion Score for dairy cows. The highest number of corpora lutea was observed after 20 and 30 days postpartum in experimental and control groups. More *corpora lutea* were observed in multiparous cows. Higher numbers of corpora lutea with similar average size were observed in right ovaries of cows in both groups, while corpora lutea were bigger in multiparous cows. Numbers and sizes of corpora lutea, may give an insight in quality of restoration of ovarian cyclicity and a solid base for prediction on future reproductive performances.

Keywords: *corpora lutea*, puerperium, ovulation, dairy cows

Introduction

To be able to sustain next gestation, bovine reproductive system should normally recover in 45 days after calving (*Brick, 2011*). Uterus in cows undergoes involution process during puerperium phase, while ovaries restore suppressed cyclicity as a result of gestation. *Corpus luteum* (CL) of pregnancy is regressed due to prostaglandin (PGF) secretion from endometrium, which causes luteolysis (*Senger 2003; Silvia et al.,1991*). After 10-20 days postpartum (PP) luteinizing hormone (LH) levels begin to raise (*Saacke et al., 2000*) and commencing of cyclicity after parturition is probably the most important factor for cows to successfully conceive again (*Mather et al.,1981*). Usually 3-4 weeks pass before first postpartum ovulation in dairy cows, often without signs of estrus. Almost 95% of dairy cows should restore ovarian cyclicity up to 50 days postpartum.

Ovulation is enabled only if dominant follicle (DF) produces enough estradiol to stimulate LH surge (*Noakes,2009*, *Rutigliano et al.,2008*). Increase of estradiol 17β (E2) secretion is followed with follicular growth and LH surge is required for ovulation (*Kesler et al.,1979*). LH surge is important for ovulation and luteinisation of granulosa and theca cells into luteal cells and proliferation of blood vessels (*Lee et al.,1985, Mutevelić et al.,2003*). Formed CL continue to grow up to 8th day of estral cycle (*Santschi et al.,2011*). Average time to first ovulation for Holstein breed is three weeks (20.8±13.2 days) (*Benmrad et al., 1986*; *Fonseca at al.,1983*) and after that, luteal phase may begin. Up to 79% of newly formed corpora lutea have central vacuole of 2-10 mm in diameter, filled with fluid (*Fricke 2002; Ginther, 1998*).

Most important factors that affect puerperium are: age, breed, season, nutrition, milking, body condition, phase of lactation, metabolic and health status (*Vasconelos et al.,1999*) and it is well known fact that stress and pain have negative effects on reproductive hormones through hypothalamus-pituitary-ovary relationship. *Garbarino* (*2004*) observed a delayed ovarian cyclicity in cows with lameness in first 35 days in milk have delayed ovarian cyclicity, which could be prevented up to 71% if lameness was absent.

According to *Patton et al. (2006)* time to first postpartum ovulation and CL forming is the shortest when milking is done only once daily. Cows with positive energy balance (PEB) have higher concentrations of circulating IGF-I, because milking once daily increase insulin and glucose concentrations. Those cows loose less weight and and keep higher body condition scores (BCS) through entire lactation, then cows milked twice or three times daily (*Patton et al., 2006*). Absence or shortening of traditional dry period cause increase of IGF-I and insulin concentrations, which trigger more frequent LH surge and earlier cyclicity (*Feu et al., 2009; Gumen et al., 2005; Walch et al., 2008*). Due to improvement of energy status in early puerperium, ovulations and more double ovulations happens earlier and more frequent (*Gumen et al., 2005; Santschi et al., 2011*), which supports importance of nutrition for productive and reproductive efficiency (*Diskin et al., 2003*). Nutritional deficiencies and negative energy balance (NEB) early PP have a serious effect to size and future of DF, since smaller follicles secrete less estradiol, which consequently have less feedback for LH secretion (*Diskin et al., 2003*). Approxemately 80% of dairy cows after parturition enter the NEB period in early lactation and BCS drops as well, because energy requirements for milk synthesis are not supported by desirable nutrition, which later compromise reproductive performances (*Domecq et al., 1997; Feu et al., 2009;, Montiel et al., 2005*). Cows with expressed NEB in first 9 days of puerperium have more negative effects to luteal function, which is usually not observed before 50-70 days in milk, when programs of artificial insemination begins (*Pankovski et al., 1995*). *Petterson at al.* (*2006*) reported that only 70.4% of Swedish Holstein cows enter normal cyclicity up to 56 days in milk.

Comparative ovary diagnostics in cows before and after slaughter, showed that 35% of diagnosis were incorrect, while failure to detect CL in various phases was estimated at 15% (*Descoteaux et al., 2006*).

Using transrectal examination *Gumen et al.* (*2003*) observed anovulatory condition in 28% of primiparous cows in period between 47-60 days in milk, while that condition was detected in 15% of multiparous cows. Rapid restoration of ovarial cyclicity after parturition is essential to achieve the best productive capacity of dairy cows (*Holt et al., 1989*), which is challenging for dairy management since it was estimated that 50% of dairy cows have irregular ovary function after parturition (*Bisinotto et al., 2010*). Longer anovulatory condition PP may be due to retained placenta (RP), metritis, slower uterine involution, longer luteal phase, low BCS, etc. (*Holt et al., 1989*). *Garmo* (*2009*) suggested that longer luteal phase could be more uterine-related problem than an issue of ovarian pathology.

The aim of this research was to explore number, size and distribution of corpora lutea in ovaries of cows with normal and abnormal puerperium status by use of transrectal palpation and ultrasonography with regard to cows' BCS and lameness status.

Material and methods

The study was conducted during winter and spring seasons of 2013. In total, the study involved 54 Holstein-Friesian cows during first 40 days of lactation, which were selected for the study based on their previous parturition dynamics data and randomly assigned to experimental (EG) (n=28) and control (CG) (n=26) group. Cows in EG received one injection of 86 μg of GnRH (Fertagyl®, Gonadorelin, Merck) at 15 days postpartum to stimulate ovarian function. All the cows were kept under the same nutritive regime, milked three times daily, and housed in a tie-stall system with separated bearings. Also, all the cows were regularly vaccinated against coronavirus and rotavirus infections and tested on brucellosis, leucosis and tuberculosis. Cows in experimental group have received one injection of 86 μg GnRH (Fertagyl®, Gonadorelin, Merck) at 15 days postpartum to stimulate ovarian function.

According to previous anamnestic data, EG and CG cows were further divided in subgroups: normal puerperium, abnormal puerperium, primiparous and multiparous cows. BCS and lameness score were estimated prior to parturition. Later BCS was monitored throughout puerperium period. Lameness was assessed using „Zinpro Locomotion Scoring“ (ZLS) for dairy cattle, while BCS was estimated by ultrasound measurement of subcutaneous adipose backfat thickness in sacro-gluteal area.

Ovary examinations were carried out every 5 days during the period from 10 to 40 days postpartum, every 5 days, which has included 378 transrectal examinations.

Ovaries and *corpora lutea* were first palpated and then observed using MyLab®30 VETGold portable ultrasound linear scanner with endorectal linear probe LV 513, 5-7.5 MHz (both Esaote SpA, Italy) and same equipment was used for monitoring of BCS.

Statistical analyses and graphical presentations of results were done by Excel® 2010 (Microsoft Inc., USA). Average observed values of CL diameter and adipose tissue thickness used to estimate BSC between the experimental cow groups and subgroups were compared using two-way Student’s t-test. The observed differences were significant if p-value was less than 5% ($p<0.05$).

Results

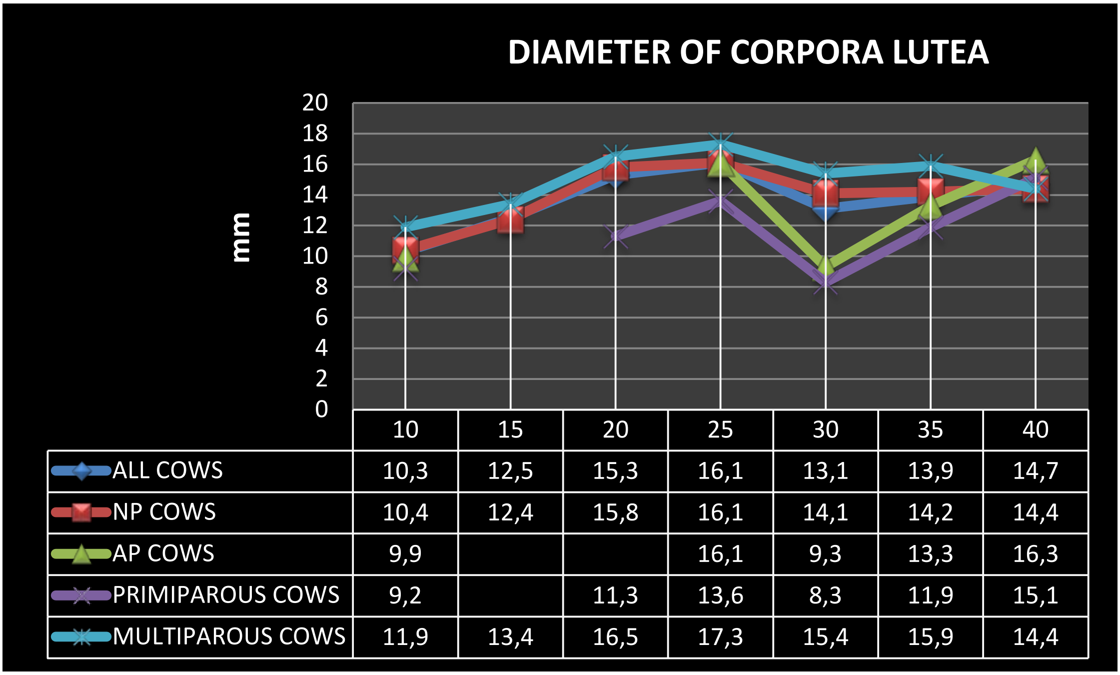
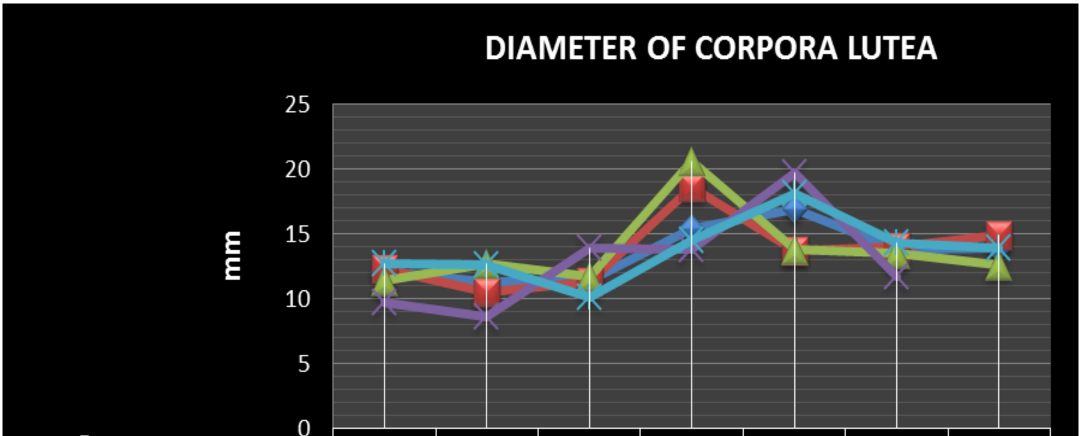


Chart 1. Average diameter (mm) of corpora lutea in experimental cow group during first 40 days postpartum. Measurements at 10 days postpartum represent remainings of gestation CL and were not included in later analysis. Statistically significant differences ($p<0.05$) between primiparous and multiparous cows were observed at 20 and 30 days postpartum

Table 1. Total number of corpora lutea in experimental group according to period and subgroups.

DAYS PP	NP COWS	AP COWS	PRIMIPAROUS COWS	MULTIPAROUS COWS
10				
15	2			2
20	9		2	9
25	7	2	2	5
30	8	3	4	7
35	7	3	4	7
40	6	3	3	6



Days postpartum	10	15	20	25	30	35	40
ALL COWS	12,1	11,3	11,4	15,4	16,9	13,8	13,9
NP COWS	12,3	10,5	11,3	18,6	13,7	14	14,9
AP COWS	11,4	12,7	11,7	20,6	13,8	13,5	12,6
PRIMIPAROUS COWS	9,7	8,6	13,9	13,8	19,7	11,7	
MULTIPAROUS COWS	12,7	12,6	10,1	14,5	18,1	14,3	13,9

Chart 2. Average diameter (mm) of corpora lutea in control cow group during first 40 days postpartum. Measurements at 10 days postpartum represents remainings of gestation CL and were not included in later analysis. Statistically significant difference ($p<0,05$) between NP and AP cows was visible only at 40 days postpartum.

Table 2. Total number of corpora lutea in control group according to period and subgroups.

DAYS PP	NP COWS	AP COWS	PRIMIPAROUS COWS	MULTIPAROUS COWS
10				
15	8			8
20	7			7
25	8	5	4	8
30	8	5	5	9
35	7	6	4	9
40	4	5		9

Table 3. Total number and diameter of corpora lutea detected by transrectal palpation and ultrasonography.

GROUP	Transrectal palpation	Transrectal ultrasonography	CL diameter (mean±st.dev)
Experimental	38	51	15.05±0.4 mm
Control	46	63	15.52±0.5 mm

Table 4. Maximal diameter values (mm) of *corpora lutea* measured by transrectal ultrasonography from 10 to 40 days postpartum (PP) in experimental cow group.

EXAMINATION DAY	NP COWS	AP COWS	PRIMIPAROUS COWS	MULTIPAROUS COWS
10				
15	11	14.1	14.1	11
20	22	12.1	12.1	22
25	24	16.1	16.1	24
30	25	12.6	12.6	25
35	19.6	15.4	15.4	19.6
40	22	16.3	20	22

Table 5. Maximal diameter values (mm) of *corpora lutea* measured by transrectal ultrasonography from 10 to 40 days postpartum (PP) in control cow group.

EXAMINATION DAY	NP COWS	AP COWS	PRIMIPAROUS COWS	MULTIPAROUS COWS
10				
15	17	22	11.5	22
20	21	19	21	12.6
25	20	23	20	23
30	33	21.4	22.4	33
35	25	17.3	15.2	25
40	22.5	15.4		22.5

Table 6. Total number of *corpora lutea* in groups with appearance of central vacuola/lacuna.

Group	Total number of *corpora lutea*	Presence of *vacuola/lacuna*
Experimental	51	25.5%
Control	63	46.03%

Table 7. Frequency of detection of *corpora lutea* and respective ovulation

GROUP	LEFT OVARY CL	RIGHT OVARY CL	OVULATION
EXPERIMENTAL (n=28)	35.4%	64.6%	74.3%
CONTROL (n=26)	40.1%	59.9%	85.0%

Table 8. Assessment of lameness in all animals at dairy farm, according to ZLS.

ZINPRO LOCOMOTION SCORE					
ZLS	1	2	3	4	5
% cows	1.1	59.2	36.3	3.1	0.3

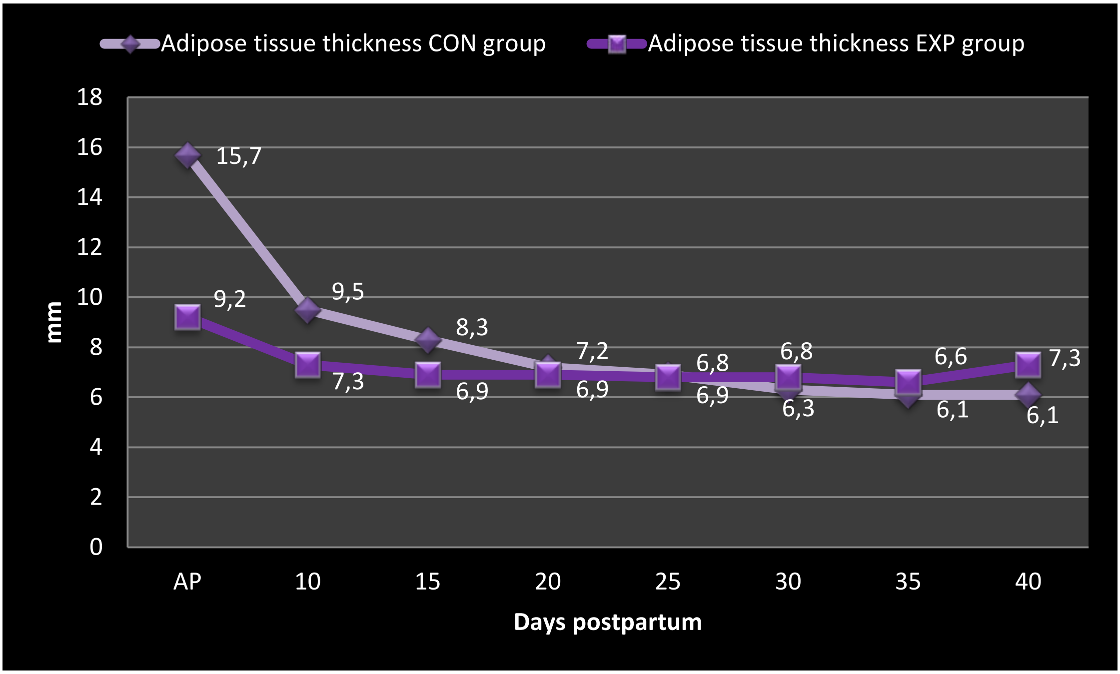


Chart 3. Average adipose backfat thickness used to assess BCS between groups during antepartum (AP) and postpartum period. Signifficant differences (p<0.05) between groups are visible only in antepartum period.

Figure 1. Sonogram: clearly visible large corpus luteum with central vacuola/lacuna 11.2 mm in diameter.

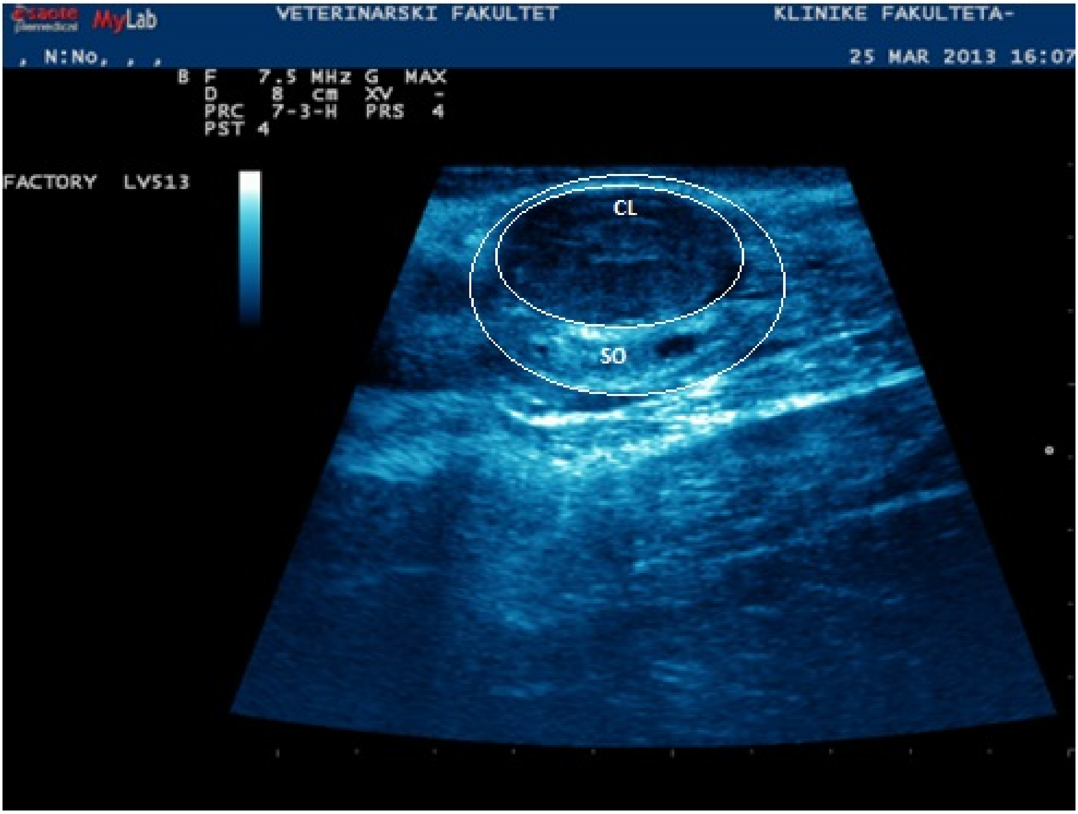


Figure 2. Sonogram: visible compact corpus luteum (CL) and ovarian stroma (SO).

Discussion

Total number of observed CL was highest in the period 20-30 days PP in EG and CG, while detection rate of central vacuola/lacuna in them was 25.5% in EG and 46.03% in CG, which fall in a range of results of other authors (*Fricke, 2002*; *Ginther*, *1998*). In EG, CL number among NP cows was 2.7 times higher than in AP cows and two times higher in multiparous cows compared to primiparous cows. In CG, total CL number in NP cows was 1.6 times higher than in AP cows, and 1.7 times higher in multiparous than in primiparous cows as well. In EG and CG cows, more CL were observed in right ovaries, while differences between groups were about 5%.

Observed CL size depended on growth or regression phase. Average CL diameter in both EG and CG was estimated at approximately 1.5 cm in average, which suggests that CL size in puerperium is not maximal, as previously described by others (*Kamimura et al., 1993*), and that CL size is larger in third than in first two postpartum ovulations as well.

Release of LH necessary for ovulation is delayed in cases of low energy intake and BCS, high milk yield and stress (*Stevenson, 2001*), which was mostly present in both cow groups, asevident by CL absence in several subgroups. Prolonged anovulatory condition PP is commonly caused by RP and metritis (*Harrison et al., 1986*), which is in line with anamnestic data in our study. According to the available dairy records, the most common puerperal disorders were RP and metritis, particularly in primiparous cows, in which anovulatory condition were most prevalent. Statistically significant differences between EG and CG were not common and observed only at 20 days PP between multiparous cows and at 30 days PP between primiparous and multiparous cows within EG.

Restoration of ovarian cyclicity largely depends on lactation number. Other authors (*Zhang et al., 2010*) reported that primiparous cows have longer acyclic periods, which corresponds with our results. In EG, 74.3% of cows displayed ovulation up to 40 days PP, while cows without ovulation were mostly primiparous with AP condition. In CG, 85% of cows showed ovulation up to 40 days PP, where cows without ovulation were again primiparous cows. Research in North American and Belgian Holstein herds (*Rhodes et al., 2003*) reported that about 23% and 38% cows, respectively manifested anestrus condition up to 60 days PP. Our explanation for such a high number of anestrus conditions could be a high number of primiparous cows in herd, especially those with AP condition, which coincide with our results. Primiparous cows have a higher incidence of delayed cyclicity than older cows, which requires more time for first luteal activity and prolongs the time from partus to first ovulatory estrus (*Petersson, 2007*). Higher risk for delayed luteolysis or cyclic abnormalities is due to low BCS up to 70 days PP, lameness, early luteal activity, metritis, RP, dystocia and abnormal vaginal discharge. Our study showed that the most of the studied cows had a low BCS during antepartum period and puerperium. Considering significant initial difference in BCS between EG and CG cows in antepartum period, our suggestion is that could be result of uneven food distribution among cows in different production phases, while additional stress to health status was present with moderate to severe lameness in 95% of all cows in a farm.

Application of GnRH is usually used to prevent delay of first ovulation in cows with AP condition (*Benmrad, 1986*), However, another opinions (*Chebel et al., 2010; Lee et al., 1985*) argue that an early restoration of cyclicity may have more positive or negative effects to fertility, because early luteal activity may have negative effects on uterine involution due to P4 secretion, which is also a risk factor for irregular luteal activity (*Garmo et al., 200;, Sakaguchi et al., 2004*). Delayed cyclicity is more common in cows with tie-stall housing system, which usually have a longer period to first ovulation and ovulatory estrus (*Petersson, 2007*), as it coincides with the farm used in our study.

Anovulatory frequency in primiparous EG cows was 33.6% and16.6% in CG, while multiparous cows in both groups displayed ovulation before 40 days PP. *Gumen et al.* (*2005*) reported that up to 28% primiparous cows within 47-60 days in milk, may have an anovulatory condition, while it was observed in multiparous cows only at 15%, which is relatively similar to our results in primiparous, but not in multiparous cows. As explained by several other authors (*Garmo et al, 2009; Holtet al., 1989; Santos et al., 2004*), a prolonged luteal phase could be more related to uterine than ovarian disorders, what was obviously reflected in detrimental effect more in primiparous cows in EG and CG, as supported by findings of *Rhodes et al.* (*2003)* and *Santos (2009*).

Seasonality plays a significant role in restoration of cyclicity by means of athmospheric and nutritional factors (*Petersson K.J., 2007; Reksen et al., 2002*). Cows calved in winter may be in higher risk of delayed and abnormal cyclicity than those calved in summer. Having in mind that all EG and CG cows in our study were calved in February and March, such a seasonal effect could be one of the reasons for a higher number of anestrus primiparous cows. *Benmrad et al.* (*1986*) reported that approximately 24% cows showed anovulation condition at least 6 weeks PP, which is close to our results in EG, but not in CG, where anovulation condition up to 40 days in milk was present only in 15 % of cows, and such a difference could be explained by more multiparous cows in CG. In addition, more expressed anovulation in EG could be explained by a higher number of primiparous cows and more frequent puerperium abnormalities like RP, metritis and mastitis, as well as low BCS and lameness in those animals.

Conclusion

- The highest number of ovulations and CL could be expected in the period 20-30 days postpartum.
- Higher number of CL was found in cows with normal puerperium and multiparous cows.
- Average size of CL is smaller during puerperium phase.
- Bigger CL were detected after GnRh administration, but only in multiparous cows.
- Multiparous cows had a larger CL, than primiparous cows.
- Frequent appearance of central vacuole/lacuna inside CL, suggests its common formation, particularly in multiparous cows.

- Body condition score and lameness, had more effect in size and number of CL in primiparous cows.
- Number and size of CL, may give an insight in quality of restoration of ovarian cyclicity and present a solid base for predicting of future reproductive performances in dairy cows

Raspodela i veličina žutih tela u mlečnih krava tokom puerperija

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Rezime

Kod krava u toku puerperalne faze materica prolazi kroz proces involucije, dok jajnici obnavljaju svoju cikličnu aktivnost, potisnutu gravidnošću.Nakon 10-20 dana postpartum (PP), nivoi luteinizirajućeg hormona (LH) počinju rasti i obnova cikličnosti je verovatno najbitniji faktor kako bi krave mogle uspešno koncipirati nakon partusa. Do 50 dana postpartum, skoro 95% mlečnih krava bi trebalo da obnovi ovarijalnu cikličnost. LH talas je važan za ovulaciju i luteinizaciju granuloza i teka ćelija u lutealne ćelije i proliferaciju krvnih sudova. Novoformirana žuta tela i do 79% imaju centralnu šupljinu, ispunjenu tečnošću. Istraživanje je uključivalo ukupno 54 Holštajn-Frizijske krave, tokom prvih 40 dana laktacije. Pregledi jajnika su rađeni u periodu 10 do 40 dana postpartum, svakih 5 dana, do kraja prvih 40 dana puerperija. Jajnici i žuta tela su prvo palpirani a zatim posmatrani upotrebom dijagnostičkog ultrazvuka „Esaote MYLAB30 VETGOLD“ sa endorektalnom linearnom sondom LV 513, 5-7.5 MHz. Ista oprema je korišćena za posmatranje BCS, dok je šepavost procenjivana upotrebom „Zinpro Locomotion Score“ za mlečne krave. Najveći broj žutih tela su uočena sa 20 i 30 dana postpartum u eksperimentalnoj i kontrolnoj grupi. Više žutih tela je uočeno u višetelki. Desni jajnici u obe grupe su imali više žutih tela, prosečna veličina je bila slična, a veći su bili prisutni u višetelki. Brojnost i veličina žutih tela može dati uvid u kvalitet obnove cikličnosti jajnika, što može biti solidna osnova za predviđanje budućih reproduktivnih performansi.

Ključne reči: žuta tela, puerperium, ovulacija, mlečne krave

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