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### PROPYLENE GLYCOL ENERGY SUPPLEMENTATION DURING PERIPARTAL PERIOD IN DAIRY COWS AND REPRODUCTION EFFICIENCY PARAMETERS

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*The aim of this work was to investigate the impact of two energy supplements based on propylene glycol in dairy cows diet on ovarian and follicular morphology, conception, insemination index and length of service period. A total number of 60 Holstein Friesian dairy cows, parity between 2-8, with an average milk production of 7000 kg/305 days of lactation were divided into three experimental groups (20 dairy cows per group). The first group of dairy cows was supplemented daily with "Energy-plus" (O1 group; 200 mL propylene-glycol supplement) and the second group was supplemented with "Ketal" (O2 group; 160 mL propylene-glycol supplement), two weeks before partus until 30 days post partum. The third experimental group were non supplemented dairy cows (O3, control group). Ultrasound examination of the reproductive system using real time echo camera Falco VET 100 (ESAOTE PieMedical, Holland, B-shaped scan with linear-array endorectal 5–8 MHz probe) was conducted on every animal starting from day 40 postpartum. The diameters of the ovaries (left and right) and of the dominant follicle(s) were recorded. Ultrasound testing was repeated on day 50 and 60 postpartum only in cows which in the meantime were not inseminated. Reproduction efficiency parameters (conception rate, number of inseminations and length of service period) were recorded individually. The statistical significance of the differences between groups was tested using ANOVA with LSD test at the level of significance  $p < 0.05$ , chi-square test and Kaplan-Meier survival analysis (the length of service period).*

*There was no significant impact of the propylene glycol supplementation on the ovarian and follicular morphology at the first ultrasound examination. At the second ultrasound examination there was a significant difference between left ovarian dominant follicle diameter in the control and supplemented dairy cows ( $1.67 \pm 0.53$  vs  $1.12 \pm 0.29$  and  $1.11 \pm 0.35$  cm,  $p < 0.05$ , O3 vs O1 and O2, respectively). The cumulative percentage of conception after the first*

and second insemination was 60%, 81% and 25% in groups O1, O2 and O3, respectively ( $p < 0.05$ , chi-square test). The insemination index was lowest in group O2 and statistically significant differences were found between groups O2 and O3 ( $1.69 \pm 0.79$  :  $3.38 \pm 1.36$ , respectively,  $p < 0.05$ ). The length of the service period was significantly ( $p < 0.05$ , LSD test) shorter in the O2 group ( $100 \pm 35$  days) compared to groups O1 and O3 ( $168 \pm 59$  and  $157 \pm 52$  days, respectively), that was confirmed by the Kaplan-Meier survival analysis of days open periods.

The use of propylene glycol supplement in dairy cows diet during peripartal period induced higher percentage of pregnancy rates after the first and second insemination, significantly shortened the length of the service period and reduced the insemination index.

Key words: dairy cows, propilen glycol, peripartal period, reproduction efficiency

#### INTRODUCTION

The early lactation period is characterized by a negative energy balance (NEB), which is primarily caused by an inadequate relationship between the requirements for milk production and feeding opportunities (appetite-ingestion) (Bell, 1995). Fertility of dairy cows reflects the cumulative effect of metabolic, endocrine, and health components that are modified by the intensive selection for high milk yield (Jorritsma et al., 2003).

Shortly after calving, between days 7-14 post partum, the follicle-stimulating hormone (FSH) level in dairy cows begins to increase. Successful development of follicles depends, besides the effects of FSH, also on the luteinizing hormone (LH) pulse frequency (Roche et al, 1998). For cows that have passed the lowest level of NEB, the return of physiological LH pulse frequency is recognized as a sign of the establishment of normal postpartum ovarian activity (Canfield et al., 1990). It is assumed that NEB does not interfere with follicular waves during the early postpartum period and follicular dynamics is a response to increased blood concentrations of FSH, which is synchronized to the end of pregnancy. In the first weeks of lactation, there is insufficient LH, but not FSH secretion, and therefore NEB does not affect the regular start of follicular growth, which depends only on FSH (Beam et al., 1997; Pate, 1999; Savio et al., 1990).

During the postpartum period the reproductive system of dairy cows undergoes major changes. After calving the uterus involutes, and the hypothalamus-pituitary-gonadal axis restarts estrus cyclicity, that practically "resets" the reproductive system (Pate, 1999). The development of dominant follicles in dairy cows occurs in waves and the majority of the estrus cycles consist of two to three such waves. Follicular waves usually occur on the 2<sup>nd</sup> and 9<sup>th</sup> day of the estrus cycle, or on the 2<sup>nd</sup>, 9<sup>th</sup> and 16<sup>th</sup> day, depending on the number of waves (Sirois et al., 1988). At the time around calving the ovarian follicles in cows are not larger than 5 mm in diameter. During the first 25 days after calving, concurrent to the reduction in energy deficit the number of Class 3 follicles (10-

15 mm in diameter) increases. Class 1 and 2 follicles (3-5 and 6-9 mm in diameter, respectively) shift from lower to upper class (Lucy et al., 2007). The final diameter of the dominant follicle and the production of  $17\beta$ -estradiol are influenced by metabolic factors. As the energy balance improved after the lowest level of NEB there is improvement in growth of dominant follicles and higher levels of  $17\beta$ -estradiol were detected (Beam et al., 1997; 1998).

It is generally accepted that in order to achieve optimal economic performance one dairy cow should have a calf every year. This goal can be achieved with successful conception before day 85. postpartum. It is also important that the early return of normal ovarian cycle (follicular and luteal phase of estrus cycle) is associated with visible signs of estrus (Opsomer et al., 1998). The early occurrence of estrus is important because in cows up to the 60<sup>th</sup> day postpartum there is a negative correlation between the number of cycles and the length of the service period (Canfield & Butler, 1990). The timing of the first estrus after calving is very variable and it usually occurs between days 30-80 postpartum. The fact is that first visible signs of estrus are not a reliable signs of early ovarian activity. Allrich (1994) showed that the first postpartum ovulation in dairy cows is usually "quiet", with no visible signs of estrus. It is the influence of progesterone on the brain structures that leads to visible external symptoms of estrus. Progesterone is secreted by the *corpus luteum*, formed after the first postpartal ovulation, and this steroid hormone makes the brain susceptible to estradiol effects during the next follicular phase of the estrus cycle.

Propylene glycol (propadiol-1,2 or propane-1,2-diol; PG) is a sweet, hygroscopic, viscous liquid with glucoplastic properties in dairy cows. It is routinely used *per os* in the treatment of ketosis, with an assumption that it can quickly increase the blood glucose level (Butler et al., 2006; Rizos et al., 2008). After absorption from the digestive system most of the PG enters the liver via the portal vein. In the hepatocytes, PG is metabolized by the lactate aldehyde pathway, into lacto-aldehyde which is then oxidized to lactate. Further oxidation of lactate to pyruvate is followed by the formation of oxaloacetate (OA), which is the starting compound in the process of gluconeogenesis (Veltman et al., 1998, Grummer et al., 1994). The oral use of PG during the postpartal period is one way to overcome the NEB and reduce the likelihood of ketosis and not weaken the reproductive function (Studer et al., 1993, Grummer, 1995). In cows treated with PG the first ovulation occurred earlier than expected and the length of the first luteal phase was within physiological limits. In the control group of dairy cows active luteal phase after the first postpartal ovulation lasts only 7.3 days, indicating a short and infertile estrous cycle (Miyoshi et al., 2001).

The service period represents the time from calving to successful conception. The average length of service period for dairy cows is between 80-90 days (Chebel, 2008). Early postpartum insemination increases the number of calves and reproductive efficiency, but it requires a higher number of semen doses used per successful pregnancy. Chabel (2008) also indicated that the first postpartum insemination may be practiced as early as 40 days after calving with an acceptable reproductive performance. The aim of this work was to investigate the impact of two energy supplements based on propylene glycol in dairy cows

diet on the ovarian and follicular morphology, conception, insemination index and length of service period.

#### MATERIAL AND METHODS

The total number of 60 Holstein Friesian dairy cows, parity between 2-8, with an average milk production of 7000 kg/305 days of lactation were divided into three experimental groups. The first group of dairy cows was supplemented daily with "Energy-plus" (O1 group, 200 mL propylene-glycol supplement) and the second group was supplemented with "Ketal" (O2 group, 160 mL propylene-glycol supplement), two weeks before partus until 30 days postpartum. The third experimental group represented the non supplemented dairy cows (O3, control group).

Ultrasound examination of the reproductive system using *real time* echo camera *Falco VET 100* (ESAOTE PieMedical, Holland, B-shaped scan with linear-array endorectal 5–8 MHz probe) was conducted at least twice on every animal starting from day 40 *postpartum*. The diameters of ovaries (left and right) and dominant follicle(s) were recorded. Ultrasound testing was repeated on day 50 or 60 *postpartum* only in those cows which in the meantime were not inseminated. Pregnancy was verified by ultrasound examination 45 to 60 days following insemination. Reproduction efficiency parameters (conception rate, number of inseminations and length of service period) were recorded individually. The statistical significance of the differences between groups were tested using ANOVA with LSD test at the level of significance  $p < 0.05$ , chi-square test ( $p < 0.05$ ) and Kaplan-Meier survival analysis (the length of service period).

#### RESULTS

The results of ovarian (left and right) and dominant follicle diameter recordings at the first and second ultrasound examinations are shown in Table 1.

Table 1. Ovarian (left and right) and dominant follicle diameter recordings ( $\bar{x} \pm SD$ , cm) at the first and second ultrasound examination

Echo.	Group	Right ovary	Left ovary	Right ovary follicle	Left ovary follicle
I	O1	2.73±0.71	2.76±0.66	1.16±0.38	1.18±0.36
	O2	2.48±0.38	2.62±0.55	1.15±0.53	1.04±0.26
	O3	2.90±0.73	2.66±0.66	1.22±0.35	1.17±0.37
	mean	2.68±0.63	2.68±0.61	1.17±0.42	1.14±0.33
II	O1	2.71±0.54	2.71±0.42	1.29±0.27	1.12±0.29a
	O2	2.86±0.80	2.95±0.58	1.28±0.33	1.11±0.35b
	O3	3.04±0.71	2.96±0.60	0.97±0.24	1.67±0.53 <sup>a,b</sup>
	mean	2.87±0.69	2.87±0.54	1.18±0.31	1.23±0.42

The results in Table 1 indicate that mean values for the right ovary and follicle diameters measured 40 days post partum were  $2.68 \pm 0.63$  and  $1.17 \pm 0.42$  cm, respectively. At the day 50 postpartum the mean values for the right ovary and follicle diameters were  $2.87 \pm 0.69$  and  $1.18 \pm 0.31$  cm, respectively. There were no statistically significant differences between the mean right and left ovary and right ovary dominant follicles diameter between experimental groups. The left ovary diameter mean values were not significantly different between groups at the second ultrasound examination. However, the left ovary dominant follicle diameter mean value in the control group of dairy cows ( $1.67 \pm 0.53$  cm, O3) at second ultrasound examination was significantly higher ( $p < 0.05$ ) compared to the other experimental groups ( $1.12 \pm 0.29$  and  $1.11 \pm 0.35$  cm, O1 and O2, respectively).

The conception rates results of experimental groups of dairy cows are shown in Table 2.

Table 2. Conception rates results (total and relative number of inseminated and pregnant cows per experimental group)

Group	Number of cows per group	Number of inseminated cows		Number of pregnant cows	
	N	Number	%	Number	%
O1	20	17	85	15	88
O2	20	20	100	16	80
O3	20	19	95	16	84
Total	60	56	93	47	84

The total number of cows that were inseminated was 56, representing 93% of all experimental animals, and 84% of those cows were pregnant after 1-5 inseminations. The results of pregnancy rate after first, second and further inseminations and statistical significance of the differences between groups are presented in Figure 1.

The results of pregnancy rate presented in the Figure 1 indicate that the majority of cows treated with energy supplements based on PG conceived after the first and second postpartal insemination. Cumulative pregnancy rate after the first and second insemination in the first and second experimental group was 60% and 81%, respectively. In the nonsupplemented control group 12 out of 16 cows (75%) conceived after three or more inseminations. There is a statistically significant difference in the proportions of pregnant animals after the first, second and  $\geq 3$  inseminations between two supplemented groups of dairy cows and the control group of animals (40%:20%:40% and 50%:31%:19% vs 12%:12%:75%; O1 and O2 vs. O3, respectively,  $p < 0.05$ , chi-square test).

The results of the average number of semen doses (insemination index) per pregnant dairy cow are presented in Table 3.

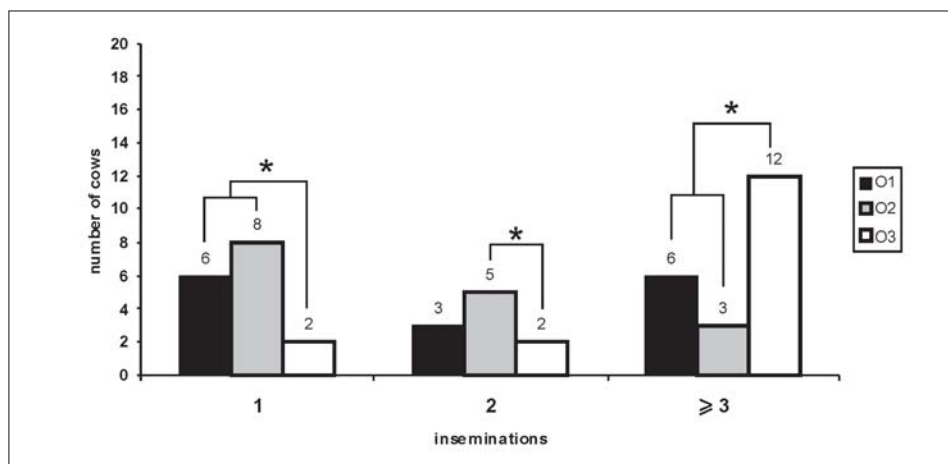


Figure 1. Pregnancy rate per experimental group according to insemination; 1 – cows recorded pregnant after the first artificial insemination, 2 – cows recorded pregnant after the second artificial insemination, ≥ 3 – cows recorded pregnant after third or more inseminations; \*– statistically significant difference between proportions ( $p < 0.05$ , chi-square test)

Table 3. The average number of semen doses per pregnant dairy cow (insemination index)

Group	n	$\bar{x}$	SD	Min	Max	CV%
O1	15	2.60	1.80	1	6	69.41
O2	16	1.69 <sup>a</sup>	0.79	1	3	47.00
O3	16	3.38 <sup>a</sup>	1.36	1	5	40.30
Total	47	2.55	1.52	1	6	59.35

Legend: n – number of pregnant cows; <sup>a</sup>Groups labeled with the same superscript are statistically different ( $p < 0.05$ , LSD test).

The insemination index represents the ratio between the total number of inseminations and the number of pregnant cows. The insemination index was  $2.60 \pm 1.80$ ,  $1.69 \pm 0.79$  and  $3.38 \pm 1.36$  (for O1, O2 and O3 group, respectively). There was a statistically significant difference in the insemination index between groups O2 and O3 of dairy cows ( $p < 0.05$ ; LSD test).

The results of the average number of days from calving to conception (service period, days open) are shown in Table 4.

The duration of the service period was  $168.47 \pm 59.20$ ,  $100.47 \pm 35.09$  and  $157.25 \pm 51.50$  days (O1, O2 and O3 group, respectively). According to the obtained results there was a statistically significant difference between the first and second, and between the second and third experimental group ( $p < 0.05$ , LSD

test). The results of Kaplan Meier survival analysis have also indicated a statistically significant difference between the second experimental group (O2, supplemented with Ketal) compared to two other experimental groups (see Figure 2).

Table 4. Number of days from calving to conception (service period, days open)

Group	n	$\bar{x}$	SD	Min	Max	CV%
O1	15	168.47 <sup>a</sup>	59.20	66	250	35.14
O2	15	100.47 <sup>a,b</sup>	35.09	62	177	34.93
O3	16	157.25 <sup>b</sup>	51.50	37	234	32.77
Total	46	142.35	56.99	37	250	40.03

Legend: <sup>a,b</sup> - Groups labeled with the same superscript are statistically different ( $p < 0.05$ , LSD test).

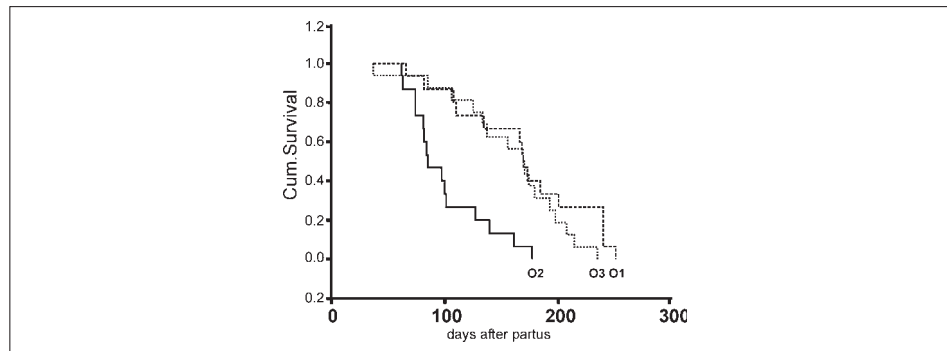


Figure 2. The results of Kaplan Meier survival analysis of days open period in all three groups of dairy cows

The results of Kaplan Meier survival analysis of days open period in all three experimental groups (Figure 2) indicate that dairy cows that were supplemented with "Ketal" (O2 group) had significantly shorter days open period compared to the other two experimental groups (O1 and O3), since the cumulative survival curves for O2 group does not cross the survival curves for two other experimental groups.

## DISCUSSION

Energy balance in lactating dairy cows can be defined as the difference between utilisable energy from dietary intake and the energy expended for body maintenance and milk production (Beam *et al.*, 1999). Parturition represents an abrupt change from pregnancy to lactation. During pregnancy the energy input is spent on foetal growth and development, as well as body reserves accretion.

However, at the onset of lactation tremendous metabolic demands of high milk production are supported by rapid mobilisation of lipid and protein body stores (Bauman & Currie, 1980; Butler, 2000). Since dry matter intake (DMI) in high producing dairy cows at the early lactation is insufficient for dramatic increase of milk production, dairy cows typically experience NEB. The NEB is characterised by a loss of body reserves and may persist for 10-12 weeks of lactation (Bauman and Currie, 1980), being most intensive in the first week of lactation (Tamminga *et al.*, 1997). During the first 3-4 weeks postpartum NEB is highly positively correlated with the interval to first ovulation (Butler, 2000). However, follicular growth and recruitment of a dominant follicle (DF) seems to be independent of energy balance (Beam *et al.*, 1997). Furthermore, PG supplementation during early lactation had no effects on follicular dynamics, mean days to emergence of the first cohort of follicles postpartum, or days to dominance and duration of dominance for any follicular wave recorded postpartum (Rizos *et al.*, 2008). Our results regarding ovarian and follicular morphology indicate no statistically significant difference between ovarian and DF diameter between PG supplemented and control group of dairy cows at the first ultrasound examination (Table 1). Surprisingly, we have recorded significantly higher left ovarian DF follicle diameter in the control group of dairy cows at the second ultrasound examination compared to the supplemented cows. Since the control group dairy cows conception occurred mostly after the third or more AI (75% of pregnancies after  $\geq 3$  AI), when energy balance was corrected. This could explain the difference in left ovary follicular diameter. However, because of the relatively small number of experimental animals this problem needs further research. There was no significant difference in the right and left ovarian diameters between supplemented and control dairy cows.

Permanent decline of modern high-milking dairy cows reproductive efficiency has been documented by many researchers during the last decades (Roche, 2000; Lucy, 2001; Dobson *et al.*, 2007). A significant worldwide decline in conception to first service in Holstein dairy cows has been recorded over the past 30 years (Beam *et al.*, 1999; Royal *et al.*, 2000). Decrease of reproductive efficiency is also evident as prolonged postpartal anoestrus (Thacher *et al.*, 2006), increase number of cows in silent estrus with irregular estrous cycle duration, resulting from short luteal phase in the first few postpartal cycles (Miyoshi *et al.*, 2001), decline in the first insemination conception rate (CR) (Lucy, 2001), as well as an increase number of cows with abnormal early embryonic development and various forms of uterine diseases, increasing embryonic and fetal mortality rate (Bouchard *et al.*, 2003; Lucy, 2007). The final result is a decline in the reproductive efficiency with an increase of number of inseminations needed for successful conception (insemination index) (Sheldon and Dobson, 2003). It was reported that successful CR after the first insemination decreased between 1990-2000 in most European countries from 55% to 45% (Bousquet *et al.*, 2000). This situation had a direct influence on the increase of the number of inseminations and Lucy (2001) reported that 20 years earlier approximately 1.75 inseminations were needed for successful conception while in the last few years it has increased to more than 3. This is important parameter of reproduction efficiency since it is



inversely proportional to the reproduction efficiency and directly proportional to DO period (Esslemont *et al.*, 2000). Our results indicate that CR at the first and second AI in supplemented dairy cows was significantly increased compared to the control group of animals (Table 3). Furthermore, cumulative pregnancy rate for first and second AI was 60% and 81% for supplemented inseminated animals. These results confirm the thesis of Chabel (2008) that first postpartum insemination may be practiced as early as 40 days after calving with an acceptable reproductive performance.

The pregnancy rate is a very significant parameter of reproductive efficiency. The energy supplement based on PG given in the peripartal (transition) period increased the number of cows which conceived after the first and second insemination (Figure 1). Miyoshi *et al.* (2001) reported successful conception in 57.1% of PG supplemented cows, and 33% in the control group. In our study only the first and second insemination may be linked to the influence of PG as it was applied up to the 30th day after calving. Boland *et al.* (1999) reported the indicators of optimal reproduction are those where 60% of cows conceived after the first insemination. The insemination index was lowest for the group of cows treated with Ketal (O2), indicating that the use of different type of PG supplement could result in the decreased number of inseminations per pregnancy.

The service period was reduced in O2 group of supplemented dairy cows (Table 4). It was significantly shorter indicating that Ketal supplementation could reduce the duration of the service period (Figure 2). This is in disagreement with the results of Miyoshi *et al.* (2001), who has reported that dietary supplementation of dairy cows with PG did not affect the duration of the service period. Most authors agree that the use of energy supplements based on PG does not improve reproductive characteristics of dairy cows (Formigoni *et al.*, 1996; Miyoshi *et al.*, 2001; Hoedemaker *et al.*, 2004; Chagas *et al.*, 2007). Our results showed that some reproductive efficiency parameters could be improved by certain energy supplements. However, the sole application of PG supplement is probably not sufficient to eliminate all negative impacts of the energy deficit on reproductive efficiency. Only adequate nutrition and housing, with all the other necessary animals health and welfare control programs as a part of modern farm management, can provide a high milk production without a negative impact on reproduction efficiency in dairy cows.

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**ENERGETSKA SUPLEMENTACIJA PROPILEN GLIKOLOM TOKOM  
PERIPARTALNOG PERIODA I PARAMETRI EFIKASNOSTI REPRODUKCIJE KOD  
MLEČNIH KRAVA**

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SADRŽAJ

Cilj ovog rada je bio da se ispita uticaj primene dva tipa energetskih dodataka u ishrani mlečnih krava na bazi propilen glikola na morfologiju jajnika i folikula, koncepciju, indeks osemenjavanja i dužinu servis perioda. U ogled je bilo uključeno ukupno 60 krava Holštajn Frizijske rase, u toku 2-8 laktacije, sa pro-

sečnom proizvodnjom mleka od 7000 kg/305 dana laktacije, koje su bile podeljene u tri ogledne grupe (20 krava po grupi). Prva grupa mlečnih krava (O1) je svakodnevno suplementirana peroralnom aplikacijom 200 mL "Energy-plus", druga grupa krava (O2) suplementirana je sa 160 mL "Ketal-a", počevši od dve nedelje pre partusa do 30 dana nakon partusa. Treću grupu mlečnih krava (O3, kontrola) su predstavljale netretirane životinje. Ultrazvučni pregled reproduktivnog sistema vršen je sa uređajem Falco VET 100 (ESAOTE PieMedical, Holland), linearnom endorektalnom sondom od 5–8 MHz kod svake životinje najmanje dva puta počevši od 40 dana posle partusa. Ultrazvučni pregled je ponovljen sa 50 i/ili 60 dana posle partusa kod krava koje nisu u međuvremenu osemenjene. U toku svakog pregleda su registrovani prečnik oba jajnika i dominantnog folikula. Kod svake ogledne životinje su određeni parametri efikasnosti reprodukcije: stepen koncepcije, indeks osemenjavanja i servis period. Statistička značajnost razlika srednjih vrednosti dobijenih parametara određivana je ANOVA metodom i LSD testom na nivou značajnosti od  $p < 0.05$ , hi-kvadrat testom i Kaplan-Majerovom analizom (dužina servis perioda).

Rezultati prvog ultrazvučnog preleda su ukazali da nema statistički značajnih razlika u morfologiji jajnika i dominantnog folikula između suplementiranih grupa u odnosu na kontrolnu grupu krava. Rezultati drugog ultrazvučnog pregleda su ukazali da postoji statistički značajna razlika između srednjih vrednosti prečnika dominantnog folikula na levom jajniku kod kontrolne grupe krava u odnosu na suplementirane životinje ( $1,67 \pm 0,53$ ;  $1,12 \pm 0,29$  i  $1,11 \pm 0,35$  cm,  $p < 0.05$ , O3:O1 i O2).

Zbirne vrednosti procenta koncepcije nakon prvog i drugog osemenjavanja bile su statistički značajno više kod suplementiranih krava u odnosu na kontrolnu grupu životinja (60% : 81% : 25%; O1:O2:O3,  $p < 0,05$ , hi-kvadrat test). Najniža vrednost indeksa osemenjavanja je utvrđena kod ogledne grupe krava suplentirane Ketal-om ( $1.69 \pm 0.79$ ), koja je bila značajno viša u odnosu na kontrolnu grupu krava ( $3.38 \pm 1.36$ ,  $p < 0,05$ ). Dužina servis perioda je bila najmanja kod grupe krava suplementiranih Ketal-om ( $100 \pm 35$  days) i značajno se razlikovala u odnosu na ostale ogledne grupe ( $168 \pm 59$  i  $157 \pm 52$  dana, O1 i O2), što je potvrđeno Kaplan Majerovom analizom.

Energetska suplementacija mlečnih krava propilen glikolom tokom peripartalnog perioda može dovesti do povećanja procenta gravidnih krava nakon prvog i drugog osemenjavanja, smanjenja vrednosti indeksa osemenjavanja i skraćanja dužine servis perioda.