The objective of this study was to evaluate the effect of a second prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) treatment during Ovsynch on luteal regression and fertility in dairy cows, compared with standard Ovsynch. The study was conducted on 111 Holstein Friesian multiparous cows on commercial dairy farm. The cows in the experimental group (n=48) received two treatments of PGF$_{2\alpha}$ 24 hours apart during Ovsynch. The cows in the control group (n=63) were synchronized with standard Ovsynch. To assess the progesterone (P$_4$) concentration blood samples were collected at the day of PGF$_{2\alpha}$ treatment and at the 2$^{nd}$ GnRH treatment. Pregnancy was evaluated by ultrasound examination 37-40 days after timed artificial insemination (TAI) by ultrasound. Cows diagnosed pregnant were re-examined between days 70-80 after TAI. The percentage of cows with complete corpus luteum (CL) regression (P$_4<$0.5 ng/ml at the time of the 2$^{nd}$ GnRH treatment) was 89.6 % after two PGF$_{2\alpha}$ treatments and 88.9 % after one PGF$_{2\alpha}$ treatment. There were no statistically significant differences (p>0.05) in the pregnancies per artificial insemination (P/AI) between the experimental and control group (P/AI). However, the pregnancy loss rate was lower in cows receiving two PGF$_{2\alpha}$ treatments than in the control animals (0.0 % vs. 6.4 %; p<0.05). In conclusion, the second PGF$_{2\alpha}$ treatment during Ovsynch protocol had no significant effect on CL regression and P/AI in dairy cows. The pregnancy losses until days 75-80 after TAI were significantly lower after two PGF$_{2\alpha}$ treatments than after one PGF$_{2\alpha}$ treatment.

**Key words:** cows, Ovsynch, second PGF$_{2\alpha}$ treatment, fertility