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EFFECTS OF PROSTAGLANDIN AND HCG ON OUT OF SEASON OESTROUS SYNCHRONIZATION AND FERTILITY AND ASSESSMENT OF PROGESTERONE CONCENTRATION FOR EARLY PREGNANCY DIAGNOSIS IN EWES

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Abstract

The study investigates the possibility of oestrus synchronization in ewes of MIS sheep population using prostaglandin (PG) outside the breeding season, serum progesterone concentration for pregnancy diagnosis and effect of hCG (human chorionic gonadotropin) on reproductive parameters. Prostaglandin was used to induce oestrus at the dose of 2.5 ml/ewe, administered intramuscularly in two injections (with 11 days interval). Responsive ewes were bred to rams and were divided into two groups, one of which was administered with hCG at the dose of 300 IU i/m 7th day post mating and the second group which was used as a control. Serum progesterone was measured on the day of prostaglandin administration and 17 days post mating. Oestrus response was 66.67%, lambing rate was 70%, average litter size was 1.7 lambs/ewe and twinning rate was 60%. Administration of hCG 7 days post mating did not improve fertility results. Lambing, prolificacy and twinning rates were higher in control than in hCG group, but no statistical difference was found between these groups. From a total of 20 ewes that were mated after PG treatment in 18 of them (90%), on the basis of progesterone concentration measured on the

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17th day after mating and subsequent lambing results, gestation was confirmed or denied.

Key words: sheep, reproduction, spring season, PGF2α, chorionic gonadotropin

1. Introduction. In most of the temperate sheep breeds, breeding and lambing patterns are seasonally reflected [1], which is why modern rearing systems use exogenous hormonal treatments for the induction/synchronization of oestrus during anoestrus period. The breeding season of sheep in Serbia starts with shortening of the day length, which is from the midsummer until the end of fall/beginning of winter. Most widely used procedure for estrus induction/synchronization is application of progestagens through vaginal sponges in combination with PMSG or FSH, especially outside the breeding season.

In addition to progestagens, prostaglandins can also be used for the same purpose. However, prostaglandins are more efficiently used for the induction/synchronization of oestrus during the natural mating season, than outside the breeding season [2]. This is because prostaglandin acts through regression of corpus luteum, thereby shortening luteolytic phase of sexual cycle [3, 4]. Therefore, it is more likely for ewes to be cyclic, with functional corpus luteum, in natural breeding season, than outside of season.

Human chorionic gonadotropin, which is similar to LH in function, may boost progesterone production [5], and stimulate conceptus growth in uterus [6, 7]. As stated by Fonseca et al. [8], administration of luteotrophic agents, such as human chorionic gonadotrophin (hCG), could provide a maternal environment more favourable to the establishment and development of the conceptus by increasing the circulating progesterone concentrations.

MIS sheep population is a meat type of sheep, of strong constitution, strong carcass conformation and good meat properties. It was obtained by using a complex crossing combination according to a precisely defined genetic procedure, using Pirot pramenka, Württemberg and Ile de France breeds. Ewes of MIS population reach sexual maturity at age of 6–8 months, which puts them in the group of fast maturing populations, with an average fertility of 130 to 160% [9].

In current practice, the off-season oestrus in ewes of MIS sheep population is synchronized using progestagens impregnated in vaginal sponges (30 mg FGA) in combined application of PMSG (750 IU), with lambing rate of about 70% and the average litter size of 2.3 [10]. However, the majority of ewes often have large litters, 3 to 4 and sometimes 5 lambs, due to the additional hormones used, which is a problem because ewes get weak during pregnancy and labour, cannot raise all lambs and lambs have low birth mass.

According to Petrović [9], occurrence of fertile estrus in MIS sheep is throughout the year, although mating season is most prominent during the fall. In such sheep populations off-season induction/synchronization of oestrus can be

achieved by prostaglandin treatment, given the possibility of cyclic activity and active CL presence in ewes [11]. Even in more seasonal sheep breed (Pramenka breed, Sjenica strain), possibility for an out of season cycling was proved by progesterone serum test and out of season breeding was induced using ram effect [12].

On the basis of these previous findings, the aim of this study was to investigate the possibility of oestrus induction/synchronization in ewes of MIS sheep population using prostaglandin outside the breeding season, serum progesterone concentration for pregnancy diagnosis and effect of hCG on reproductive parameters.

2. Material and methods. Research was carried out outside the breeding season, in March and April, at the experimental farm of the Institute of Animal Husbandry in Belgrade. The trial included 30 ewes of Mis sheep population, aged 3 to 5 years.

For the purpose of oestrus induction ewes received two injections of prostaglandin (Dinoprost, Animal Health–Pfizer) at doses of 2.5 ml/ewe, administered intramuscularly, 11 days apart (Fig. 1). Teaser rams were used to detect females in oestrus. Ewes that responded to treatment after the first injection, mated approximately 36–40 h later, and were kept with rams for 8–10 h. Ewes that failed to respond after first application of prostaglandin, received second injection 11 days later. Responsive ewes mated after 36–40 h. For mating, ram to ewe ratio was 1:1–2. After second PG injection ewes that have not responded were left into groups with rams to be mated naturally 17 days later.

Blood samples for analysis of progesterone concentration were taken from jugular vein, on the days of first and second prostaglandin injection, and on the 17th day post mating. Jugular blood samples were collected in tubes. After coagulation, samples were centrifuged and the blood serum was extracted. The serum was then stored at $-20\,^{\circ}\mathrm{C}$ until analyzed for the progesterone concentration by EIA progesterone tests.

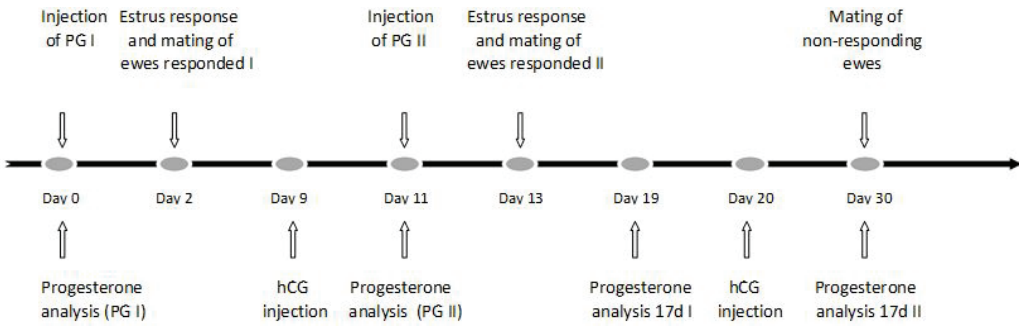

Fig. 1. Schematic presentation of PG protocol for oestrus induction/synchronization and hCG treatment in ewes during the non-breeding season

Mated ewes that responded to prostaglandin treatment were divided into two groups, one of which was administered with hCG (Pregnyl, Schering–Plough, Netherlands) (10 animals) at the dose of 300 IU i/m, on the 7th day post mating and the second group (10 animals), which was used as a control.

The following reproductive parameters were measured: Oestrus response (number of ewes showing signs of estrus/total ewes treated), lambing rate (number of ewes lambed/number of ewes mated), litter size (number of lambs born/number of ewes lambed) and twinning rate.

Statistical analysis of the experimental data was performed using the statistical package Statistica for Windows 7 (StatSoft, Inc.). The progesterone concentrations were analyzed by Mann–Whitney U-test. Data were expressed as the mean $\pm$ se. Differences between hCG and control groups concerning reproductive parameters (lambing, prolificacy and twinning rates) were tested by Chi square analysis. Analyses were performed for the significance level of 5% and 1%.

3. Results. The results of oestrous response, lambing rate and litter size of ewes following PG synchronization method are set out in Table 1.

From a total of 30 ewes in the experiment, 20 of them (66.67% of ewes showing oestrus) responded to the PG treatment and mated, 16 after the first application, and 4 after the second application of prostaglandin. Lambing rate was 70% (14/20), average litter size was 1.7 lambs/ewe and twinning rate was 60%. Ten ewes did not respond to treatment, and they were mated naturally 17 to 30 days after second PG injection. From this group, 6 ewes lambed, which is a lambing rate of 60%.

The overall lambing rate, including ewes that responded to treatment and those that were subsequently mated, was 66.67% (20/30), with an average litter size of 1.7 lambs/ewe and twinning rate of 75%.

Administration of hCG 7 days post mating did not seem to improve fertility results. Lambing rate, prolificacy rate and twinning rate were higher in control group (70%, 180% and 87.5%, respectively), than in hCG group (60%, 160% and 70%) (Fig. 2), but no statistical difference ($P > 0.05$) was found between these groups.

T a b l e 1

Effect of PG on reproductive performance of adult ewes

Treatment	No of ewes responded	No of ewes mated	No of ewes lambed	Lambing rate %	Litter size	Twinning rate %
PG I + II	20 (66.6%)	20	14	70	1.7	60
After treatment (non-responsive ewes)		10	6	60	1.67	66.67
Overall		30	20	66.67	1.7	75

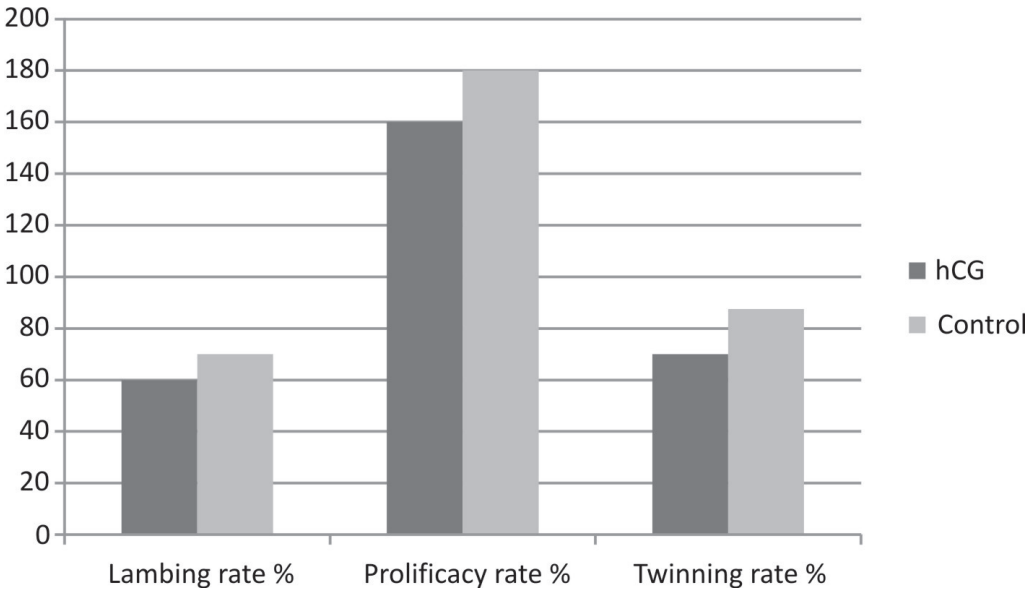

Fig. 2. Effect of hCG on reproductive performance of ewes

T a b l e 2

The mean (± se) progesterone concentrations at two blood samplings

	Sampling days			
	Day of PG injection		Day 17 after mating	
	I PG injection	II PG injection	After I PG injection	After II PG injection
Ewes responding	$0.47^{a} \pm 0.28$	0.95 ± 0.37		
Ewes not responding	$0.02^{b} \pm 0.01$	0.44 ± 0.30		
Ewes mated and lambed			$10.26^{a} \pm 3.63$	17.37 ± 4.34
Ewes mated not lambed			$0.39^{b} \pm 0.21$	

^{a,b} – means with different superscripts within a column are significantly different at $P < 0.01$
Ewes responding – ewes exhibiting oestrus after treatment and accepting mating; Ewes not responding – ewes not exhibiting oestrus after treatment and not accepting mating; Ewes mated and lambed – ewes that responded to treatment, mated and subsequently lambed; Ewes mated not lambed – ewes that responded to treatment, mated, but did not lamb;
*all ewes that mated after 2nd PG injection lambed

Table 2 shows the average values of progesterone concentration in blood serum of ewes mated after PG treatment.

Results obtained from this experiment showed that the mean progesterone concentrations on the day of PG I injection were 0.47 ng/mL for ewes that responded to treatment by exhibiting oestrus and accepting mating two days later and 0.02 ng/mL for ewes not responding to treatment, which was significantly different at $P < 0.01$. That difference was not observed on the day of PG II injection ($P > 0.05$), but mean levels of progesterone were higher (0.95 and 0.44 ng/mL, respectively).

On the 17th day post mating, the mean concentration of progesterone at first sampling (after PG I injection) was 10.26 ng/mL in ewes that later lambed and 0.39 ng/mL in ewes that were mated but did not lamb, which was significantly different at $P < 0.01$. All ewes that mated after second PG injection lambed and had mean concentration of progesterone of 17.37 ng/mL. Minimum value of serum progesterone measured in ewes determined as pregnant was 3.65 ng/mL and maximum was 34.45 ng/mL.

From a total of 20 ewes that were mated after PG treatment in 18 of them, on the basis of progesterone concentration measured 17 days post mating and subsequent lambing results, gestation was confirmed or denied, which is the accuracy of 90%. Based on this information, 12 sheep were determined to be pregnant, and 6 as non-pregnant. The two errors in the present investigation occurred with regard to ewes that were diagnosed as non-pregnant (progesterone levels 0.04 and 0.7 ng/ml), but did lamb.

4. Discussion. Fertility results from hormonally induced/synchronized oestrus vary depending on a number of factors, such as methods applied, season and type and doses of hormones used. Although use of prostaglandins for manipulation of the sexual cycle in sheep is mainly limited to natural breeding season, Yadi et al. [2] reported lambing rate of 70% and twinning rate of 42% after PG treatment in ewes outside the breeding season.

In the present study, PG treatment in ewes outside the breeding season resulted in 70% lambing rate, 1.7 lambs/ewe of average litter size and 60% of twinning rate. The overall fertility results, including ewes that immediately responded to treatment and those that were subsequently mated, were 66.67% for lambing rate, 1.7 lambs/ewe for litter size and 75% for twinning rate. This lambing rate is comparable to that obtained by Yadi et al. [2], while twinning rate was higher in the present study. Previous study by Maksimović [10], in which FGA/PMSG protocol was used to induce oestrus in ewes of MIS sheep population outside the breeding season, resulted in 69.77% lambing rate and litter size of 2.33 lambs/ewe. The present study showed the same lambing rate, while the average litter size was reduced, but no additional hormones were used.

Measurement of blood progesterone concentration is a reliable indicator of the functional corpus luteum [13]. Based on the previous study of the analysis of

progesterone profiles in the blood serum of 42 sheep of Sjenica strain [12], it was concluded that ovarian activity is lowest in March (41.67% sheep in anestrus), immediately after lambing period (January-February). However, in April, corpus luteum activity was observed in 70.37% of sheep. Most individuals had suprabasal progesterone concentration values (2–3 ng/ml in 37.04% of sheep), while 33.33% of sheep had a progesterone concentration of more than 3 ng/ml. Only 6 of 27 sheep (22.22%) had progesterone levels below 2 ng/ml, indicating on anestrus or oestrus [12].

As mechanism of prostaglandin action requires active CL presence, serum progesterone concentration should be elevated at the time of PG application. As stated by Nasar and Rahman [14], blood levels of progesterone are low at oestrus (less than 1.0 ng/ml) through to day 3 of dioestrus, and then rapidly increase to maximal levels at day 8, and remain elevated until days 11–12. However, in the present study, initial levels of serum progesterone, measured at the time of PG injection, were not indicative of CL presence, but ewes that responded to treatment had higher serum progesterone level (0.47 and 0.95 ng/mL) than non-responsive ewes (0.02 and 0.44 ng/mL). This difference was found to be significant ($P < 0.01$) at the time of first blood sampling (day of PG I injection). The difference remained at the time of second PG injection, but it was not significant ($P > 0.05$).

Progesterone, which is also known as pregnancy hormone, is useful tool for early pregnancy diagnosis in sheep. Various authors reported different threshold progesterone values to discriminate between pregnant and non-pregnant animals, ranging from 0.5 to 2.5 ng/mL in early pregnancy [15–17]. Yotov [18] reported threshold value for single pregnancies to be less than 8.8 ng/mL and more than 15.1 ng/mL for twin pregnancies on the 20th day post mating. In the present study, the mean concentrations of progesterone on the 17th day after mating were 10.26 ng/mL (I sampling) and 17.37 ng/mL (II sampling) in ewes that were found pregnant and later lambed, with minimum recorded value of 3.65 and maximum of 34.45 ng/mL. Ewes that were detected as non-pregnant had mean serum progesterone of 0.39 ng/mL, which was significantly lower ($P < 0.01$) than in pregnant ewes. This finding of significant differences ($P < 0.01$) in the concentrations of serum progesterone between pregnant and non-pregnant ewes is in accordance with previous finding of Boscos et al. [16] and Karen et al. [17]. It seems that even suprabasal level of progesterone can be enough for sheep synchronization with PG in breeds prone to out of season mating.

From a total of 20 ewes that were mated after PG treatment in 18 of them, on the basis of progesterone concentration measured 17 days after mating and subsequent lambing results, gestation was confirmed or denied, which was the accuracy of 90%. This is in agreement with results of Boscos et al. [16] who reported the accuracy of 91.4% for pregnancy diagnosis by the means of progesterone concentration measured 19 days after sponge removal and mating of ewes.

Yotov [18] reported lower accuracy level of 76.9% on the 20th day post mating, while Karen et al. [17] found 81.6% accuracy for positive predictive diagnosis and 100% accuracy for negative predictive diagnosis.

Human chorionic gonadotropin (hCG) is known by its role in suppressing early embryonic loss through increase of progesterone concentration in livestock, when administered in early stages of pregnancy. However, although previous studies reported the embryotrophic effect of hCG in ewes [6, 7] and increase in lambing rates and fetal size [19] in the present study, lambing rate and litter size were not increased by hCG administration in ewes. Zamiri and Hosseini [20] suggested that hCG is not a viable source of gonadotropin for this type of synchronization system, which is in accordance with the results obtained in the present study. Also, these results confirm previous findings of Yates et al. [5] about the absence of influence of hCG on lambing rate or number of offspring at parturition.

5. Conclusions. Results of this study suggest that prostaglandins could be used for oestrus induction outside the breeding season in ewes of MIS sheep population. hCG administration 7 days post mating had no effect on reproductive performance. Plasma progesterone evaluation is reliable tool for pregnancy diagnosis in ewes. It is costly, time-consuming procedure, but in the absence of satisfactory early clinical test in sheep and variable efficiency of oestrus detection under field condition, plasma progesterone evaluation is useful for early pregnancy diagnosis.

Considerations of cost, time required for laboratory procedures, and the need for blood sampling at known times after breeding are factors which, at present, limit the usefulness of plasma progesterone evaluation for pregnancy diagnosis, except for research purposes. In sheep, however, no satisfactory early clinical test exists, and detection of oestrus, as an indicator of failure to conceive, is of variable efficiency under field conditions.

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