Slovak J. Anim. Sci., 42, 2009, SUPPLEMENT 1: 30-34

ISSN 1337-9984

Scientific paper formerly presented

THE EFFECT OF MELATONIN, ITS COMBINATION WITH FGA AND ECG AND OVSYNCH PROTOCOL ON THE LEVELS OF STEROID HORMONE AND MORPHOMETRY OF OVARIES DURING OESTRUS SEASON OF EWES

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ABSTRACT

The effect of 1) melatonin (MEL) alone; 2) its combination with intravaginal sponges (40 mg FGA) and with 500 IU eCG and 3) OvSynch Protocol on the concentrations of progesterone (P_4), oestradiol 17β (E_2) in the blood serum, morphometry of ovaries and population of follicles during physiological oestrus of sheep was studied. Experimental groups 1 (n=6) and 2 (n=5) were administered MEL before the start of oestrus season (5 mg per head/day) for thirty days. After 30 days of MEL administration the intravaginal sponges were instilled to the group 2 for 12 days. After removal of sponges eCG was injected. The ewes of the group 3 (n=11) were treated according to OvSynch. The concentrations of P_4 and E_2 were determined in the blood serum using the RIA. The ovaries were fixed in 4 % formalin after ovariectomy and consecutively processed by standard histological methods. The results were statistically processed and evaluated by ANOVA. The significant differences were recorded after comparison of the concentrations of P_4 ($P < 0.05$) and E_2 ($P < 0.01$) between group 1 and group 3. The morphometric parameters as well as the representation of follicles population showed improvement, but difference between the experimental groups which was in favour of group 2 was non-significant. Treatment with MEL alone induces the cyclic function of ovaries, but it was not able to synchronize the ovarian processes. Its combination with other environmental methods is more advantageous. OvSynch shows positive oestrus synchronizing effect at taking into consideration species characteristics of the cyclic function of ovaries.

Key words: melatonin; sheep; ovaries; steroid hormone

INTRODUCTION

Melatonin is referred to as the "hormone of darkness" for its diurnal rhythm of secretion and release from the epiphysis during night, too. Its actions as chemical stimulation photoperiodically mediate secretion of hormones from the hypophysis (Chemineau, 1992; Thiery et al., 2002). In sheep besides the acceleration of their sexual maturity, in autumn season at shortening of the light day and simultaneous lengthening of darkness it indicates the start of sexual season – physiological oestrus.

Preparations on the basis of progestagens imitate the function of *corpus luteum* producing progesterone. They are characterised by a high activity already at a small dose. A degree of resulting induction and synchronisation as well as an interval between the treatment finishing and oestrus onset depends on the pharmaceutical agents used (Cognie, 1990; Haresign, 1992, Godfrey et al., 1999).

The combination of gonadotropins with prostaglandins is used in the breeding practice as OvSynch protocol – ovulation synchronising protocol, in most cases for cattle (Evans et al., 2004; Deligianis et al., 2005).

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This work was supported by the project AV 4/0113/06.

The goal of our work was to observe and compare the effect of exogenous administration of melatonin alone, and its combination with the application of vaginal sponge impregnated with fluorogestonacetate (FGA) and equine chorioic gonadotropin (eCG) and the treatment according to OvSynch protocol (combination of gonadotrophin releasing hormone and prostaglandin $F_{2\alpha}$) on the concentrations of ovarian hormones (progesterone – P4, 17β-estradiol –E2) and IGF I in the blood serum, morphometric parameters (length, width) and representation of follicle population of the sheep ovaries during physiological oestrus season (autumn, September).

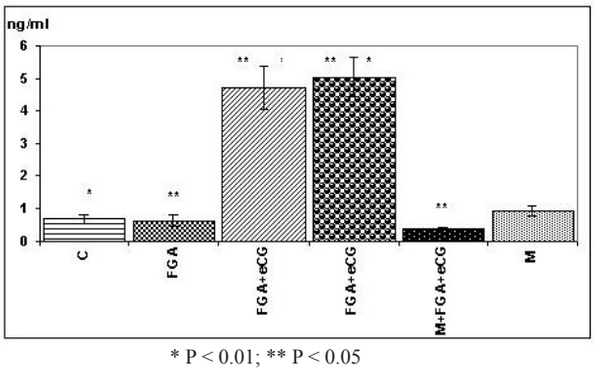
MATERIAL AND METHODS

The experiment was carried out in the agricultural farm in the sub-mountain region of the Low Tatras. Tsigai ewes (n=22) aged 4–6 years having mean live weight of 45–50 kg and body condition score of 2.5–3.5 were divided into three experimental groups according to the planned treatment. The ewes of the experimental groups 1 (n=6) and 2 (n=5) were administered MEL before the start of oestrus season (August) at a daily dose of 5 mg per animal for thirty days. Intravaginal sponges containing 40 mg of fluorogestonacetate (FGA, Chrono-gest, Intervet International B.V., Boxmeer, the Netherlands) were instilled to the animals of the group 2 (n=5) for 12 days after finishing MEL application.. At the time of their withdrawal the ewes were administered 500 I.U. of equine chorioic gonadotropin (eCG, Sergon a.u.v., Bioveta, Inc., Ivanovice na Hané, Czech republic). The third experimental group (n=11) was treated according to Ovsynch protocol: 0.5 ml GnRH/animal (Lecireline, Supergestran a.u.v., Ferring-Léčiva, Czech republic (ChR); after five days 0.5 ml $PGF_{2\alpha}$/animal (Cloprostenolum, Oestrophan inj. a.u.v., Bioveta, Ivanovice na Hané, ChR); after 48 h again 0.5 ml GnRH/head. Blood was collected for determination of steroid hormones (P_4; E_2) 48 hours after eCG administration. After ovariectomy sheep ovaries were evaluated morphometrically, fixed in 4 % formalin and processed by standard histological methods. Blood was centrifuged at 2000xg, at a temperature of 4 °C for 15 minutes. Serum was frozen until further processing. The concentrations of progesterone and 17β-estradiol were determined using the radioimmunological competitive analysis with commercial sets RIA Progesterone and RIA Estradiol (Immunotech Sa, Beckman Coulter Company, Marseille Cedex, France) in the blood serum. Results were statistically processed and evaluated by the ANOVA test. They are presented as the values of the arithmetical means and their standard error (S.E.M.).

RESULTS

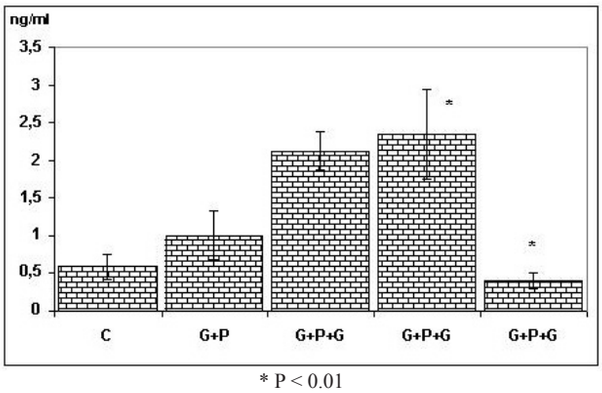
The concentrations of P_4 in the blood serum in the group 1 were on the basal level just prior to the treatment. After 12 days from instillation of sponges and before injection of eCG for influence of synthetic gestagen we registered inexpressive decrease in its concentration. The application of eCG injection induced a significant increase in P_4 ($P<0.05$). A significant decrease in P_4 ($P<0.05$) was registered for the combination of melatonin with intravaginal sponges and eCG. Melatonin alone showed similar results, but the levels were a bit higher.

The effect of the treatment according to OvSynch protocol showed a progressive increase in the P_4 concentration in the blood serum with significance in the period of finishing the experiment ($P<0.01$).



* P < 0.01; ** P < 0.05

Fig. 1: The concentrations of progesterone in sheep blood serum in relation to different treatments

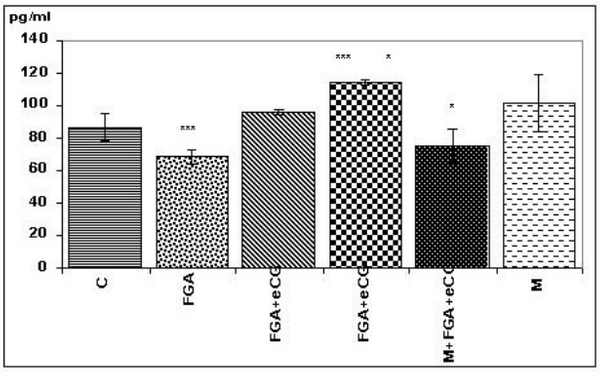


* P < 0.01

Fig. 2: The concentrations of progesterone in sheep blood serum according to OvSynch protocol (prosim vymenit cisla napr. 3,5 ako 3.5 po lavej strane v Y-osi)

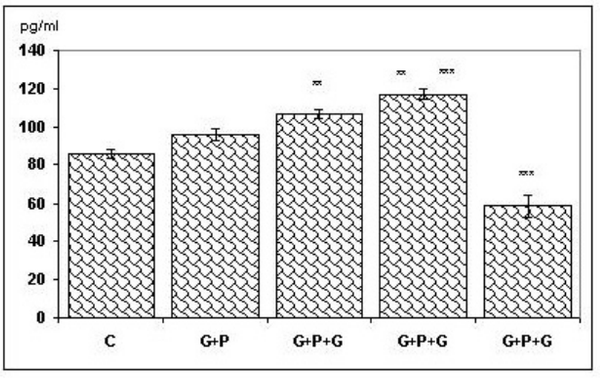
The analysis of the results of 17β-estradiol concentration showed that after application of FGA it decreased in the blood serum in comparison with the control concentrations (before treatment). After eCG injection its

concentration showed a progressive significant increase (P<0.001). A significant decrease was observed (P<0.01) for the combination of this treatment with melatonin. In the group where sheep were administered Melatonin alone the levels of 17β-estradiol were similar to those as after the treatment with FGA + eCG. The combination of gonadotropin with prostaglandin demonstrated similar changes as progesterone. Both the significant increase (P<0.05) at the time of hormonal preparation and the significant decrease (P<0.001) at the time of finishing the experiment were observed.



* P < 0.01; *** P < 0.001

Fig. 3: The concentrations of oestradiol 17β in sheep blood serum in relation to different treatments



* P < 0.05; *** P < 0.001

Fig. 4: The concentrations of oestradiol 17β in sheep blood serum according to OvSynch protocol

In addition to the levels of steroid ovarian hormones we evaluated the morphometric parameters of left and right ovaries (length, width) in cm. The comparison of the morphometric results of left and right ovaries revealed the differences to the benefit of those in the group with combination treatment, thus FGA+eCG as OvSynch (GnRH+$PGF_{2\alpha}$+GnRH).

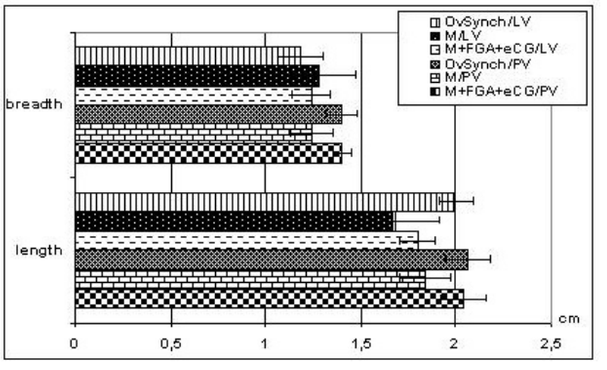


Fig. 5: Biometric parameters of sheep ovaries in relation to different treatments (prosim vymenit cisla napr. 0,5 ako 0.5 po lavej strane v X-osi)

The total count of follicles and their representations were evaluated on the individual ovaries, and the maximal follicle was determined. The groups without any treatment combination of hormonal preparations showed lower total number of follicles. A similar picture was observed in the size categories – smaller than 3 mm and bigger than 3 mm. Most of the follicles bigger than 5 mm were found on the right ovaries in the group with combination treatment; the biggest follicle found was 8.6 mm.

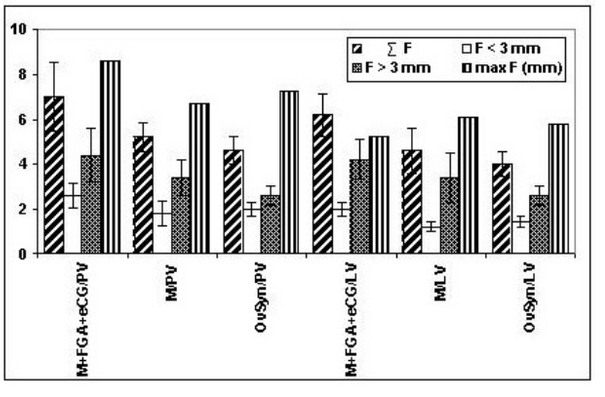


Fig. 6: There presentadion of follides in sheep ovaries in response to different treatments

DISCUSSION

The reproduction functions in sheep are influenced by the neuroendocrine changes in the concentrations of melatonin. The effect of photoperiodism manifests in variation of its levels. Melatonin increases duration of its secretion proportionally to a decrease in the number of light hours. This leads to the stimulation of the neurons

producing GnRH, and subsequently secretion of GnRH, LH and FSH is triggered. The seasonal changes in the reproduction activity are in response to it (Malpaux et al., 1997; Thiery et al., 2002). Exogenous melatonin application shows to be a suitable preparation for the control and induction of oestrus because it simulates day shortening. Its application is possible during thirty and more days in the form of aural implants with gradual release of melatonin. Henderson and Robinson (2000) reported that the method of continual melatonin release from the implant was successfully used in combination with other environmental techniques ("ram effect", intravaginal sponges impregnated with progestagen). When the effect of melatonin with the treatment combination of gestagen and eCG is compared, the treatment with melatonin alone induces the cyclic function of ovaries, but it does not synchronise the ovarian processes. Therefore, it is not possible to plan mating season in advance independently on the oestrus symptoms (Mulsant et al., 2001; Notter et al., 2003).

At present, the method of subcutaneous implantation of tablet containing melatonin is coming into focus. Here, efficacy of substance release during 7–8 weeks and its withdrawal is not necessary (Bister et al., 1999; Mulsant et al., 2001; Ptaszynska, 2002; Notter et al., 2003). Such treatment enables positive increase in the ovulation rate (Henderson and Robinson, 2000).

Progestagens or hypophyseal inhibitors block the GnRH production as well as FSH secretion. Put of maturation of follicles, start of oestrus and ovulation sometimes bring about the regression of periodic *corpus luteum* simultaneously. Their effect manifests in prolongation of the luteal phase. The oestrus cycle is reversibly repressed and after discontinuation of release its blocking effect is on the hypothalamo-hypophysial-ovarian system, which continues. The synthetic analogs of progesterone can be applied in different forms: (1) intravaginal sponges or pesars impregnated with fluorogestonacetate (30–40 mg FGA, Chrono-gest), or medroxyprogesteroneacetate (60 mg MAP, Veramix), (2) CIDR with the content of natural progesterone, (3) aurally, subcutaneous implants etc.

The use of prostaglandins ($PGF_2\alpha$ and its synthetic analogous) as means of synchronization and ovulation induction is very effective, fast, potent and easy. Its essence is the luteolytic function of $PGF_2\alpha$ which precipitates regression of corpus luteum and shortens the luteal phase of the cycle. The animals are sensitive to $PGF_2\alpha$ only between days 5–16 (or 5–14) of the cycle. One-off treatment with prostaglandin is positively prosperous only for 70–75 % sheep. The variability of sensitive response and injection form of application can limit their use (Henderson and Robinson, 2000).

Gonadotropins can be classified as stimulators of the progress of follicles or substances inducing ovulation in anoestric or oestric ewes. Inclusion of PMSG/eCG at a dose of 300 – 500 IU during breeding season and an additional dose of 400 – 600 IU breeding season off has been recommended (Henderson and Robinson, 2000). The combination with intravaginal sponge instillation during 12 days can be regarded as common in the breeding practice. At the time of their withdrawal application of PMSG/eCG dose is recommended (Evans and Maxwell, 1987; Cognie, 1990).

At present, combination of gonadotropins with prostaglandins – OvSynch protocol in ewes starts to be introduced, too (Deligianis et al., 2005). The use of OvSynch Protocol is prospective for achievement of the positive effect of sheep oestrus synchronisation as in cyclic as acyclic animals. Davis (2004) and Deligianis et al. (2005) reported success of this method by taking note of type characteristics of cyclic and acyclic ovaries. First treatment, day 0 – start of ovulation and progress of *corpus luteum* or intrafollicular luteinization, day 5 – application of $PGF_{2\alpha}$ to induce luteolysis, 48 hours from its application, injection of GnRH to evoke the ovulation again. After artificial insemination, 36–62 hours from the second injection of GnRH, majority of animals remained pregnant.

We observed dynamic changes in the concentration of steroid ovarian hormones – progesterone and 17β-estradiol in the blood serum, which are indicators of hormonal changes before the start of the oestrus cycle and also influence of the methods used for oestrus induction in sheep.

CONCLUSION

There is only little information in literature about the effect of exogenous application of pharmacological melatonin, its combination with FGA + eCG for Slovak breeds (Tsigaia, Wallachian, Merino etc). According to our partial results as well as literary information it is possible to state that Tsigaia sheep farm in the mountain region of Slovakia are stringently seasonally determined. Based on this fact it can be stated that their oestrus cycle can be re-induced out of the breeding season only by using the hormonal preparations in combination with breeding methods such as ram introducing 48 hours from the last treatment with increase in reproduction as lactation of milking ewes. OvSynch Protocol shows less marked changes in comparison with the treatment with FGA and eCG. Despite this we can establish that this method of oestrus induction in sheep appears to be a useful resultant effect of oestrus – synchronisation for ewe breeds bred in Slovakia.

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