

Szent István University
Postgraduate School of Veterinary Science

Seasonality of reproduction in Awassi sheep
Theses of Ph.D. dissertation

Written by:

Vera Faigl, DVM

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Szent István University
Postgraduate School of Veterinary Science
Budapest, Hungary

Supervisors:

†Prof. Gyula Huszenicza Szent István University Faculty of Veterinary Science Department and Clinic of Obstetrics and Reproduction
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Prof. Sándor Cseh
Szent István University Faculty of Veterinary Science
Department and Clinic of Obstetrics and Reproduction

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Vera Faigl, DVM

INTRODUCTION

In traditional technologies, sheep' dairy products are typically seasonal goods. Due to the race's inherent seasonal reproduction activity, their manufacturing is restricted to early summer. However, great commercial interest is attached to the continuous milk production. In small ruminants, gestagen treatment is widely used for cycle induction to get out-of-season fertility, but in view of the increasing consumer requirements, it is better to refrain from the use of sexual steroids in reproduction management of milked flocks. An alternative solution for the future is to form flocks, where most of the ewes are cyclic during the whole year allowing fertilization also in spring besides autumn. Based on researches we know that the ability of perennial cyclicity is genetically determined in sheep, and is in connection with melatonin receptor 1a gene (MT1) polymorphism (Pelletier et al. 2000, Notter et al. 2003). Simultaneously numerous preconditions can influence the manifestation of this capacity: like age, body condition, or pheromone-exposure. In genotypes which are genetically capable of year-long ovarian cyclicity, the use of long-known natural or near-natural breeding technologies (flushing, pheromone exposure, photoperiodic and/or melatonin treatment) might contribute to clean, green and ethical animal breeding. Apart of the relationship between photoperiodic signal and reproductive performance, the positive link between long-day photoperiodic treatment and increased milk yield was proven in cow and goat (Dahl 2000, Mabweesh et al. 2007). Thus treatments acting through the melatonin pathway to induce cycle in the short-day breeder sheep may negatively influence milk production in lactating dams.

Rams also show year-round variation in sexual behaviour, testicular size, and quality of semen, thus to maximize the out-of-season reproductive performance also rams should be treated accordingly. Administration of melatonin in the non-breeding season was shown to influence positively the hypothalamus-pituitary-gonadal axis in rams (Kennaway and Gilmore 1985, Lincoln and Ebling 1985, Bourla 1991, Chemineau et al., 1992) and the treatment was also able to increase ram effect (Rosa et al. 2000, Abecia et al. 2006a).

The investigated Awassi breed is a fat-tail sheep which originates from the Middle East. Improved Awassi flocks are kept indoors all year round and an accelerated lambing regime is practiced with several mating/insemination periods during the year (Epstein, 1985). Ewes are milked from the first day of lactation, and lambs are removed from the dam into an artificial rearing unit after birth. Climatic and feeding conditions and also the daily and seasonal rhythm of daylight are completely different in the lowland area of the Carpathian basin than in the Middle East and the degree of seasonality of the inland population was not investigated before.

AIMS OF THE STUDIES

Our aim was to investigate the reproductive seasonality of a dairy Awassi flock in Hungary and its relation to the melatonin receptor 1a (MT1) polymorphism. Secondly we wished to test the value of hormonal and non-hormonal tools to alter seasonal variations. To have an insight on these characteristics, we aimed to perform the following experiments:

- 1) Following up of ovarian activity in the postpartum period. To determine the time of resumption of cyclicity and to identify the factors influencing it (season, age, parity, milk yield, metabolic status, as a consequence of the possible presence of negative energy balance in lactating dams: lipid mobilization, hyperketonaemia).
- 2) On the basis of data collected in the first experiment, in the second trial we tried to use one of the identified factors (day length / light exposure) to modify the length of postpartum acyclicity.
- 3) To determine the proportion of dams showing out-of-season ovarian cyclicity and to recognize the factors affecting this capability (age, parity, body weight, metabolic status, milk yield and possible impact of MT1 polymorphism).
- 4) To compare the definite efficacy of different cycle induction / synchronization protocols at different time points of the year. With special attention to the efficiency of long-lasting melatonin treatment and the so-called Ovsynch protocol.
- 5) To evaluate the effect of long-term melatonin treatment applied during non-breeding season on semen characteristics and endocrine function of testicles in Awassi rams used as semen donors in artificial insemination programs.
- 6) Finally by synthesizing the knowledge acquired through the above experiments we intended to work out a recommendation of reproductive management system (know-how) for intensive dairy Awassi flocks with focusing on the biological specialty of the given breed under continental weather.

STUDIES

Experiment 1 and 2

In two consecutive experiments seasonal differences in the resumption of postpartum ovarian activity, milk production, and the periparturient metabolic status was investigated in lactating non-suckling dairy awassi sheep. In Experiment 1 autumn lambing (AL, n=27) and spring lambing (SL, n=37) ewes were investigated. Ovarian activity was monitored by means of individual progesterone (P4) profiles from day 5 to day 100 postpartum. Most of AL dams (89%) ovulated till day 35 after parturition and became cyclic thereafter. Incidence of persistent corpus luteum (CLP) and short luteal phases (sCL) was frequent (18% and 29%, respectively) among non-conceiving dams. In contrast only 24% of SL ewes ovulated before day 35. P4 levels during luteal phase in cyclic animals were lower, and length of cycle was longer in SL compared to AL animals. No CLP or sCL was detected in the spring lambing group, 61% of SL remained acyclic till the end of trial. Lactational length was significantly longer in SL dams compared to AL (P=0.008). According to the examined plasma metabolites (BHB, NEFA) and metabolic hormones (insulin, IGF-I, thyroxin), negative energy balance did not appear in any of the animals. However seasonal differences were seen in IGF-I and thyroxin levels being higher in SL dams.

In Experiment 2 influence of additional lighting was studied in autumn lambing ewes. Long-day photoperiod (LD, n=23) group was exposed to artificial light from sunset till midnight (approx. 16 hours light / 8 hours dark) from some weeks before the expected date of delivery in mid September until the end of December. Control group (n=25) experienced only natural daylength. Time of first postpartum ovulation tended to delay in LD animals compared to Controls (P=0.047). Lactational length of LD group tended to be longer (P=0.061). NEFA, BHB, insulin, IGF-I and thyroxin levels did not differ between groups.

Conclusion

Despite the situation seen in Holstein cattle, in commercial, lactating, but non-suckling Awassi ewes we found no interaction between postpartum metabolic changes and the onset of ovarian activity. It seems that in a given animal-keeping technology, beside the suspected genetic background, the most important regulatory factor of the resumption of reproductive activity is the photoperiod.

In autumn-lambing dams first postpartum ovulation may happen before the completion of uterine involution which increases the risk of uterine infections. In autumn-lambing dams additional artificial light (approximately 16 hours light) may delay the time of first postpartum ovulation, and thus may be a suitable tool to avoid the unwanted premature ovulation. At the same time long-day photoperiodic treatment may also prevent early drying-off of autumn-lambing animals. Additional studies are needed to optimize the intensity and timing of light-treatment.

Experiment 3

The relationship between milk production, metabolic hormone levels, melatonin receptor 1a (MT1) gene polymorphism and out-of-season cyclicity was evaluated in 395 Awassi dams. Milk progesterone (P4) concentration of spring-lambing ewes was determined 3 times 7 days apart between 10-12 weeks postpartum (pp). Animals with P4 levels >4 nmol/L in at least one of the samples were considered cyclic. Body weight was recorded and plasma leptin and insulin-like growth factor I (IGF-I) concentrations were measured during the same time. Monthly records of test milking were collected from each animal during the first 3 months of lactation. MT1 polymorphism at the MnlI and RsaI RFLP sites (presence: M and R, or absence: m and r of the MnlI and RsaI restriction site, respectively) was determined.

After MnlI digestion, allele frequency was 0.55 for M and 0.45 for m; frequency of the M/M, m/m and m/m genotypes was 0.36, 0.38 and 0.26 respectively. After RsaI digestion allele frequency was 0.83 for R and 0.17 for r; frequency of the R/R, R/r and r/r genotypes was 0.70, 0.26 and 0.04 respectively. The population was in Hardy-Weinberg disequilibrium for both RFLP sites.

Milk yield, leptin, and IGF-I level significantly influenced the capability for ovarian cyclicity in the late spring – early summer period. The highest proportion of cyclic ewes were among those that had high plasma leptin levels ($P<0.0001$) and medium IGF-I concentrations ($P<0.0001$). Milk yield and date of lambing was negatively associated with cyclic ovarian activity ($P=0.001$ and $P=0.048$, respectively).

The effect of genotype on out-of-season cyclicity was not apparent in subgroups of ewes with optimal metabolic status where the proportion of cycling animals was the highest. However genotype was an important factor in animals with below-median plasma IGF-I and above-median plasma leptin level. In this subgroup, a higher proportion of ewes with R/R and M/M genotypes were cycling ($P=0.007$ and $P=0.09$, respectively).

Conclusions

Melatonin receptor 1a gene was found to be polymorphic at both the RsaI and MnlI restriction sites, with high incidence of R and M alleles in the examined Awassi flock. The ability of out-of-season cyclicity is expressed in mature animals having high plasma leptin level (indicating adequate energy stores). However, high milk production may negatively influence this behavior. Our results suggest that the preferable allele (R at a significant level and M tendentially) can enhance out-of-season cyclicity in dams with suboptimal metabolic condition related to low plasma IGF-I. Nevertheless, these polymorphisms are not suitable for marker assisted selection because only the RsaI RFLP site showed significant effect and it was limited to a subgroup of the flock. However, beside the melatonin receptor, several other biologically active components (e.g. hormones, proteins) were suggested and/or proved to play a crucial role in the neuroendocrine control of seasonal reproductive activity in sheep, of which genetic background could be interesting to investigate in our experimental flock.

Experiment 4

We compared the efficacy of three different cycle induction/synchronisation protocols used out of the breeding season in dairy Awassi ewes. In Experiment 1 (Exp.1) 85 autumn-lambing dams were used. Proportion of out-of-season cycling animals was determined by assaying milk progesteron (P4) or fecal gestagen metabolites 3 times 7 days apart on d0, d7, d13 (Exp.1 d0:10th February). The Gest group was treated in April with gestagen sponge (d56-d70) + 600IU eCG (d70). The Mel+Gest group received a melatonin implant (Melovin®, CEVA, Libourne, France) on d0 and was synchronised as the Gest group 56 days later. The Mel+GPG animals were treated with melatonin (d0) and synchronised with GnRH (d63) – PGF2 α (d70) – GnRH (d72). Ewes were inseminated twice (fixed-time AI) and were introduced to rams 14 days later. Individual P4 profiles were followed from d45 to d99. Pregnancy associated glycoprotein was assayed on d99 and d133. Date of conception was determined according to lambing dates. IGF-I and thyroxin levels were determined from all samples collected from day 46 until 28 days post AI. In Exp.2 the protocol was repeated with 115 spring-lambing dams (Exp.2 d0:22th June).

According to the detected progesterone values 39% of ewes showed cyclic ovarian function, but only 6% remained active until the end of April. There was no difference between groups in this respect. Following cycle induction/synchronization those receiving gestagen+eCG treatment ovulated in higher proportion compared to those getting long-term melatonin

implant followed by Ovsynch protocol (Gest: 96% vs Mel+Gest: 95% vs Mel+GPG: 45%; $P=0.040$). This suggests that melatonin treatment in February was too early and was not able to override photorefractoriness, thus was unable to induce cycle. In Gest and Mel+Gest groups 14% of ewes conceived from timed AI, however only 3% of Mel+GPG animals got pregnant. Rams fertilized within 1 month 10% of Gest group, 5% of Mel+Gest group and 3% of Mel+GPG (NS). At the same time 31-43% of ewes conceived several months later, at the end of summer, beginning of autumn, and 38-62% remained opened for more than 220 days. Conception rate was obviously influenced negatively by the high proportion (54%) of young ewes. According to literature data average age of puberty in Awassi ewes is 7.5 months (4-15 months) (Talafha et al. 2011) which supports our theory. Our previous research in the same flock in accordance with literature data suggests that proportion of out-of-season cycling ewes is depending on age, older dams are more likely to cycle.

In June 4% of ewes were cycling (NS). However this proportion was higher in melatonin-treated ewes between day 45-56 compared to control (19% Gest vs. 44% Mel+Gest vs. 47% Mel+GPG; $P=0.109$). The proportion of dams ovulating following synchronization was 100% in both Gest and Mel+Gest groups, however only 88% in Mel+GPG (NS). At the same time only 24% (Gest), 22% (Mel+Gest) and 5% (Mel+GPG) conceived from timed AI ($P=0.104$). Rams fertilized 65% of ewes during the same breeding season (NS). 8-27% of dams remained opened for more than 150 days following synchronization (NS).

Comparison of day of conception by survival analyses revealed no differences between groups neither in Autumn, nor in Spring lambing dams (Autumn-lambing $P=0.361$; Spring-lambing $P=0.131$).

In accordance with our expectations plasma IGF-I levels of first-parity ewes was significantly higher in all groups compared to non-lactating older dams ($P=0.001-0.095$). This difference was even more expressed in melatonin-treated ewes ($P<0.008$). Between week 6 and 10 after the insertion of melatonin implants, treated animals had lower plasma IGF-I levels in all age-groups compared to Control ($P=0.005-0.100$). These differences were equalized by the end of the experimental period. Plasma thyroxin was not affected by melatonin treatment or age.

Conclusions

Our results suggest that reproductive activity of Awassi is clearly seasonal under continental climate, and breeding season is shorter than in its homeland, at the near-east where it lasts from April until September (Zarkawi 1997). Melatonin implant used in February was not able to induce cycle, however it had beneficial effect in June. At the same time through depression of IGF-I level, melatonin treatment may negatively influence milk yield and the length of lactation. GPG protocol for synchronization can only be effective when used near to the natural breeding season and in ewes at least 2 years of age.

Experiment 5

On the 23rd of February (d0), slow release melatonin implants were inserted subcutaneously into rams (n=8). Control animals (n=8) received no treatment. In both groups, basic semen parameters (concentration, total motility, fast and slow forward motility, morphology), GnRH- induced testosterone response and basal IGF-I concentration were evaluated on d0, d47 and d71. No differences were found in concentration of spermatozoa, total motility, and numbers of spermatozoa with fast and slow progressive motility and normal/abnormal morphology between melatonin treated and control groups. However, in melatonin treated animals, basal and GnRH-induced testosterone levels were slightly elevated on d47 and became significantly higher on d71 ($P<0.05$) compared to controls. There was no difference in plasma IGF-I levels between groups.

Conclusion

The use of slow release, long acting melatonin implant in Awassi semen donor rams had positive effect on the endocrine function of testicles in February, but at the same time this beneficial effect was not reflected in semen quality. Plasma IGF-I level was not influenced by melatonin treatment. Further investigation is needed to dissolve this contradiction between exocrine and endocrine findings concerning testicles and also to study the interaction between melatonin and growth hormone - IGF-I axis in small ruminants.

NEW SCIENTIFIC RESULTS

The below results are thought to represent remarkable novelty value:

1. In non-suckling autumn-lambing Awassi dams first postpartum ovulation happens very early (89% before day 35 PP), even before the completion of uterine involution which increases the risk of uterine infections. Short luteal phases and persistent corpora lutea are frequent (irregular progesterone profiles altogether 33%) in autumn-lambing dams and a remarkable proportion (41% of the autumn-lambing ewes; 100% of those with irregular progesterone profile) remains non-pregnant following mating.
2. Contrary to the autumn-lambing ewes resumption of ovarian cyclicity happens later (only 29% before day 35 PP) in spring-lambing ewes, and irregular cycles are rare.
3. In autumn-lambing dams additional artificial light (up to 16 hours/day) delays the time of first postpartum ovulation, and thus may be a suitable tool to avoid the unwanted premature ovulation. At the same time long-day photoperiodic treatment also prevents early drying-off of autumn-lambing animals.
4. Melatonin receptor 1a gene (MT1) is polymorphic at both the RsaI and MnlI restriction sites, with high incidence of R (0.83) and M (0.55) alleles in the examined Awassi flock.
5. The preferable allele (R at a significant level and M tendentially) can enhance out-of-season cyclicity only in dams with adequate fat stores and high milk yield (suboptimal metabolic condition related to low plasma IGF-I and high plasma leptin level). Nevertheless, these polymorphisms are not suitable for marker assisted selection because only the RsaI RFLP site showed significant effect and it was limited to a subgroup of the flock.
6. When applying melatonin treatment in lactating ewes, due to the depression of IGF-I level, there is a risk of decline in milk yield and shortening of lactation. Plasma IGF-I level is not influenced by melatonin treatment in rams.

IMPLEMENTATION OF NEW SCIENTIFIC RESULTS IN DAIRY SHEEP BREEDING

Relying on the previously described series of experiment put side by side with literature data we worked out a reproduction technology to provide continuous milk production in intensive dairy Awassi flocks („know-how”). Parts of this complex system are presented here. Suggestions relying on the new findings of the present thesis are highlighted as *italic bold*:

- 1) *To provide continuous milk production the breeding technology must rely on year-round handling of two set of dams kept separately. (a) half of the flock would conceive in the traditional breeding season (September-October) from natural cycle, and lamb at the end of winter – beginning of spring; (b) other half of the flock would conceive in April following cycle induction and lamb in September.*
- 2) Animals conceiving during the natural breeding season (in autumn) and lambing at the end of winter – beginning of spring (mating between 1st of September – 25th October → pregnancy check between 5th to 20th of December):
 - This set of animal can be easily formed of (i) dams lambing in spring of the given year, and (ii) of ewes born during the autumn of the previous year or the spring of the given year (≥7 months old ewes) and having adequate body condition
 - *Ewes became spontaneously cycling at the end of August (first parity animals mainly at early September).* The onset of cyclicity may be enhanced by the following feeding and zootechnological tools: flushing and ram-effect.

Although ovarian cyclicity can be induced effectively with hormonal treatments (gestagen + eCG from mid August, *or slow release melatonin implant from the summer solicite*), the use of these treatments is not needed at a flock level, and is furthermore costly.
 - Suggested breeding technology at flock level is natural mating in harems; insemination with fresh or frozen semen should be restricted to high genetic value dams.
 - For management reasons mating should be restricted to the time interval between 1st of September and 25th of October.
 - Synchronization of cycle is needed only in cases when dams are inseminated artificially. *The recommended method of synchronization is the long-term gestagen sponge combined with eCG injection at sponge removal. If the consumer requirements desires and economical background allows, cycle synchronization*

before insemination is also possible by GnRH + PGF2 α + GnRH combination, however this method is more labor intensive and more costly compared to the gestagen + eCG protocol.

- To increase conception rate, drying off of lactating dams at early September can be considered.
- *Pregnancy check*: transabdominal ultrasonography should be performed between the 5th and 20th of December (2.5 - 3.5 MHz, B-mode, sector probe). The method allows the identification of pregnant and non-pregnant dams (from day 30 of pregnancy) and the recognition of twin pregnancy (from day 45-50)

Cautions: ***To securely use the above described management system, the mating period in autumn has to be finished approximately 45-50 days before pregnancy check, thus the breeding season should end by 25th October.***

- Lambing date of conceived ewes is expected between 1st February and 31th of March. The following breeding of dams will take place similarly, between 1th September and 25th October.

3) Animals conceiving from induced cycle (in April) and lambing in September (mating between 1st and 30th of April → pregnancy check between 15th May and 50th June):

- This set of animal can be formed of (i) dams lambed in the previous autumn, (ii) of ewes born during the end of winter – beginning of spring of the previous year (≥ 12 months old ewes) having adequate body condition, and (iii) those healthy dams which were found to be non-pregnant at the pregnancy check in december.

Cautions: technology ***will fail if the formation of the autumn-lambing flock is relying*** mostly on dams which did not conceive in the normal breeding season, ***on young ewes (<10-11 months-old), or on animals wich do not reach adequate body condition.***

- ***Most of the dams and first-parity ewes are acyclic in April. Thus cycle induction with hormonal treatment is necessary*** (12-14 day-long gestagen treatment started in the last days of March, beginning of April, and 500-600 IU eCG injection at removal). First ovulation is expected between 48-65 hours following gestagen removal. ***If the animal does not conceive from the first ovulation, one or two additional cycles can be expected, but ovarian activity will become acyclic again thereafter.***

- The success of cycle induction treatments may be enhanced by the following feeding and zootechnological tools: flushing, ram-effect.
 - Suggested breeding technology at flock level is artificial insemination with diluted fresh semen (produced locally).
 - ***Rams has to be removed from the flock 26-30 days following sponge removal.***
 - ***Pregnancy check: 45-50 days after the end of the mating period (method: same as in December).***
 - Lambing date of conceived ewes is expected in September-October.
 - ***In autumn-lambing dams the first postpartum ovulation, in part because of the early weaning, and in part because of the stimulatory photoperiodic signal, will take place soon, in many cases even before the completion of uterine involution. This presumably increases the risk of subclinical alterations of uterine involution. At the same time lactation length is shorter and re-conceiving dams dry off soon after getting pregnant. To prevent these negative effects (thus to increase lactation length and to delay the first postpartum ovulation), the following management interventions are suggested:***
 - ***Dams has to be kept separately from rams, far enough from each-other to exclude pheromone effect.***
 - ***Additional artificial lightening should be applied from lambing (beginning of September). Proportion of light and dark hours for long-day photoperiodic signal has to be 16:8, or additional lightening has to be applied during the photo-sensitive phase (between 16-18 hours after sunrise).***
 - The following breeding of dams will take place similarly, throughout April of the next year. To increase the success rate, drying off of lactating dams before starting the cycle induction treatment can be considered.
- 4) ***Determination of RsaI polymorphism of MT1 gene in all animals, and following-up of reproductive performance can provide additional information on photoperiodic sensitivity. Although in our study beneficial effect of the presence of R allele was only significant in a subset of animals with suboptimal metabolic status, further research with a higher number of sheep may justify the utility of the above mentioned mutation as a genetic marker.*** Decreased seasonality would augment the number of animals cycling throughout the year, and allow for spring mating without hormonal treatments, profiting only from the use of ram-effect and flushing.

PUBLICATIONS RELATED TO THE TOPICS OF THE PRESENT THESIS

Full text papers published in peer-reviewed journals in English:

1. **Faigl V.**, Keresztes M., Kulcsár M., Nagy S., Keresztes Zs., Amiridis, S., Solti L., Huszenicza Gy., Cseh S.: Testicular function and semen characteristics of Awassi rams treated with melatonin out of the breeding season. *Acta Vet. Hung.*, 2009. 57: 531-540. (IF: 0.642)
2. **Faigl V.**, Keresztes M., Márton A., Fébel H., Kulcsár M., Nagy S., Cseh S., Solti L., Huszenicza Gy.: Effect of season and photoperiod on the time of first postpartum ovulation in Awassi ewes. *Acta Vet Hung.*, 2011. 59(4): 497-510. (last known IF: 1.264)
3. **Faigl V.**, Keresztes M., Reiczigel J., Kulcsár M., Dankó Novotni G., Chilliard Y., Jávör A., Cseh S., Huszenicza Gy., Árnysai M.: Relationship between seasonality of reproduction, metabolic status and MT1 receptor gene polymorphism in Awassi sheep. [Submitted for publication to *Theriogenology*]
4. Cseh S., **Faigl V.**, Amiridis, G.: Semen processing and artificial insemination as part of health management of small ruminants. A review. *Anim Rep Sci.*, 2012. 130(3-4): 187-92. (last known IF: 1.721)
5. Radoslava, V., Kostecká, Z., **Faigl, V.**, Marton, A., Keresztes M., Árnysai M., Kulcsár, M., Dankó G., Svantner R., Nagy, S., Csatári G., Cseh S., Solti, L., Huszenicza Gy., Maracek, I.: Recent progress in endocrine, nutritional and genetic aspects of ovine reproduction. *Folia Veterinara (Kosice)*, 2006. 50: 157-166. (IF: 0.000)
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7. **Faigl V.**, Marton A., Keresztes M., Novotniné Dankó G., Csatári G., Antal J., Nagy S., Árnysai M., Kulcsár M., Cseh S., Huszenicza Gy.: Az anyajuhok szaporodási teljesítményének növelésével összefüggő egyes újabb élettani kérdések és ezek technológiai vonatkozásai. Irodalmi áttekintés. *Magy. Áo. Lapja*, 2005. 127: 586-593. (IF: 0.114)
8. **Faigl V.**, Keresztes M., Márton A., Schneider Z., Korvin L., Nagy S., Novotniné D. G., Árnysai M., Jávör A., Cseh S., Huszenicza Gy.: Melatonin-, ill. fénykiegészítés alapú ciklusindukciós technikák kiskérődzőkben. Élettani vonatkozások és gyakorlati alkalmazás. Irodalmi áttekintés. *Magy. Áo. Lapja*, 2007. 129. 219-230. (IF: 0.104)
9. **Faigl V.**, Keresztes M., Árnysai M., Kulcsár M., Balogh O., Nagy S., Szenci O., Cseh S., Huszenicza Gy.: Melatonin alapú ciklusindukciós technikák tejelő Awassi juhokban. *Magy. Áo. Lapja*, 2012. 134: pp. 24-29. (last known IF: 0.3)

Poster or oral presentation on international conferences:

10. **Faigl V.**, Keresztes M., Kulcsár M., Márton A., Nagy S., Cseh S., Huszenicza Gy.: Effect of melatonin treatment on plasma IGF-I and thyroxin level, and endocrin function of testicles in Awassi sheep. Magyar Buiatrikus Társaság XVIII. Nemzetközi Kongresszusa, Siófok, 2007. okt. 10-13, oral presentation
11. Cseh S., **Faigl V.**, Keresztes M., Kulcsár M., Nagy S., Amiridis G., Solti L., Huszenicza Gy.: Testicular function and semen characteristics of rams treated with melatonin out of the breeding season. 11th Annual conference of the European Society of Domestic Animal Reproduction (ESDAR), Celle, Germany. Abstract. *Reprod. Dom. Anim.*, 2007. 42. 71. oral presentation
12. **Faigl V.**, Keresztes M., Kulcsár M., Márton A., Nagy S., Dankó G., Magyar K., Solti L., Cseh S., Huszenicza Gy.: Postpartum resumption of cyclic ovarian function on non-suckling dairy (Awassi) ewes lambled in different seasons. 11th Annual conference of the European Society of Domestic Animal Reproduction (ESDAR), Celle, Germany. Abstract. *Reprod. Dom. Anim.*, 2007. 42. 140. poster presentation
13. Árnysai M., **Faigl V.**, Keresztes M., Kulcsár M., Reicziegel J., Jávör A., Cseh S., Huszenicza Gy.: Effect of MT1 gene polymorphism and some metabolic factors on out-of-season ovarian cyclicity in dairy Awassi sheep. 12th Annual conference of the European Society of Domestic Animal Reproduction (ESDAR), Utrecht, Netherland. Abstract. *Reprod. Dom. Anim.*, 2008. Vol: 43(5): 87. poster presentation
14. **Faigl V.**, Keresztes M., Árnysai M., Kulcsár M., Nagy S., Cseh S., Huszenicza Gy. Melatonin-based induction of cyclicity in intensive dairy (Awassi) flocks. International Congress on Animal Reproduction, Budapest (Hungary) 13-17 July 2008. *Reprod. Dom. Anim.*, Vol. 43. Suppl 3. page 74. Poster presentation
15. **Faigl V.**, Árnysai, M., Keresztes M., Kulcsár M., Reiczigel J., Jávör A., Cseh S., Huszenicza Gy.: Seasonality of reproduction and MT1 receptor gene polymorphism in Awassi sheep. International Congress on Animal Reproduction, Budapest (Hungary) 13-17 July 2008. (*Reprod. Dom. Anim.*, 2008. Vol. 43. Suppl 3, page 11). Oral presentation
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Vera Faigl DVM
Department and Clinic of Obstetrics and Reproduction
Faculty of Veterinary Science, Budapest