Milk progesterone profiles, blood metabolites, metabolic hormones and pregnancy rates in Awassi ewes treated by gestagen + eCG at the early breeding season

A. Marton[1,3], V. Faigl[1], M. Kerestes[1], M. Kulcsar[1], S. Nagy[2], H. Febel[4], G. Novotni Danko[5], K. Magyar[5], F. Husveth[3], L. Solti[1], S. Cseh[1], Gy. Huszenicza[1]

[1]Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary
[2]AWASSI Corporation, Bakonszeg, Hungary
[3]Georgikon Faculty, University of Pannonia, Keszthely, Hungary
[4]Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary
[5]Institute of Animal Sciences, University of Debrecen, Debrecen, Hungary

ABSTRACT: The ovarian response to a standard chronogest + eCG treatment with plasma levels of insulin, insulin-like growth factor-I (IGF-I), thyroids, non-esterified fatty acids (NEFA), βOH-butyrate (BHB) and urea-N (PUN) was studied in lactating Awassi ewes (n = 105) during the late-summer – early autumn transition period. The ewes were inseminated with diluted fresh semen after gestagen removal, and mated thereafter; 26 of them conceived at the fixed-time AI (fix AI; conception rate is calculated from lambing dates). Ovarian function was monitored by milk progesterone (P_4) profiles. Before synchronization, the ovary was still acyclic in 33 and already cyclic in 72 ewes. Twenty-nine and 43 of the cyclic animals were in the follicular and luteal phases, respectively. After gestagen removal almost all (n = 104) ewes ovulated, although at AI elevated P_4 levels related to the presence of partially luteinized follicles, and short-lived CL-s were observed in 10 and five animals (none of them re-conceived at the fixed time AI). Cycling ewes showed higher insulin and IGF-I levels than the acyclic animals, and those who had not conceived had higher PUN than the pregnant ones. The other metabolic parameters did not differ. Neither conception rate, nor the ovarian response was influenced by the pre-treatment.

Keywords: dairy ewe; cycle-induction/synchronization; ovary; insulin; IGF-I

Out-of-season induction/synchronization of ovarian cyclicity is of great importance in sheep farming, specially when using artificial insemination techniques. Synchronization of ovulation denotes the manipulation of the length of the follicular or luteal phase resulting in higher conception rate following fix timed AI or natural mating (Wildeus, 1999). Among the hormonal protocols available, in addition to prostaglandin (Harvey et al., et al., 1984; Teatcher et al., 2001; Ptaszynska, 2006) and melatonin treatment (Haresign, 1992; Chemineau et al., 1996; Ptaszynska, 2006; Chemineau et al., 2008), the most widely used technique is the gestagen + eCG (syn. PMSG) combination (Mac Donnell and Crowley, 1978; Fukui et al., 1983; Abecia et al., 2002; Emsen et al., 2003; Ucar et al., 2005). Although this latest technique is widely used, there is little data available in the literature about its effect on follicular dynamics and ovarian response in small ruminants in different cycle phases before treatment.

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An important limiting factor of the success of synchronization / induction treatments is the body condition of animals. A minimum threshold of energy stores is needed to achieve normal reproductive performance. In ruminants plasma insulin and insulin-like growth factor-I (IGF-I) are connected with energy intake and energetic status. Beside their systemic effect, insulin and IGF-I have been shown to have direct positive impact at the ovarian level as well, enhancing follicular growth and sexual steroid synthesis (Spicer et al., 1993; Spicer and Stewart, 1996). In dairy cattle higher peripheral insulin and IGF-I levels are associated with shorter postpartum acyclicity (Beam and Butler, 1997).

Objectives

Our experience with dairy Awassi ewes has shown that the date of conception following gestagen + eCG treatment varies widely among individuals (unpublished data). Monitoring ovarian activity by individual progesterone profiles drew our attention to the possibility that part of the animals may ovulate during treatment resulting in variation of conception day. The present study was designed to compare the success of synchronization treatment in cyclic and acyclic animals and also to compare animals in the follicular and luteal phases at the beginning of the synchronization treatment. The effect of metabolic state and body condition was also investigated.

MATERIAL AND METHODS

Animals

The experiment was conducted in an intensive commercial dairy flock of Awassi ewes where animals were kept in opened barns, and fed on a diet containing concentrate, fresh alfalfa and grass hay (diet before flushing; Table 1). Lambs were weaned immediately after birth and ewes were milked twice daily. Spring lambing ewes, with parity 2–10 were involved in the study (n = 108). Dams were still in milk at the beginning of the next breeding season (late August – early September), when they were sampled. Daily milk yield varied between 1.1–2.5 kg at the beginning of the experiment and subsequently did not fall below 0.5 kg. Experiments were carried out according to the animal welfare regulations of the Veterinary Faculty of Szent Istvan University.

Table 1. Daily composition of diets fed to the experimental ewes

Components	Diets	
	before/after flushing	flushing (2 weeks)
Concentrate (kg/day)	1.2	1.7
Corn (%)	47.0	37.5
Wheat (%)	31.5	36.0
Soybean meal (%)	10.0	
Lupin grain (%)		15.0
Aminoplus* (%)	4.0	4.0
Magnapac** (%)	4.0	4.0
Premix (%)	3.0	3.0
Salt (%)	0.05	0.5
Grass hay (kg/day)	0.5	0.5
Fresh alfalfa (kg/day)	2.0	2.0
DM (kg/day)	1.95	2.37
Energy (MJ NEm/day)	15.13	19.20
Protein (g/day)	340.26	425.24

DM = dry matter, NEm = net energy for maintenance
*bypass protein; **bypass fat

Experimental design

Beginning from mid August ewes were fed for 14 days with a lupin supplemented diet providing elevated energy density of the ration (flushing diet, Table 1). Ovarian cyclicity was induced/synchronized with gestagen + eCG treatment by the end of August as follows: from 22nd August to 4th September 40 mg Fluorogeston (Cronolone) sponge intravaginaly (Chrono-gest), at the time of gestagen removal of 500 IU eCG (Chrono-gest PMSG inj.; both products: Intervet International B.V., Angers, France). Day 0 (D 0) of the experiment was defined as the insertion of the gestagene sponge. Body weight and body condition score of animals was recorded before and after flushing (D –7 and D 16). Ovarian activity was monitored by individual milk progesterone profiles. Milk samples were collected twice weekly from 14 days before sponge insertion (D –14) until gestagen removal (D 14), then every

day for nine days (D 14–23), and three times weekly thereafter until D 44.

Ewes were inseminated with fresh semen 48 and 60 hours after sponge removal (fix timed AI), thereafter the ewes returning to estrus were mated with rams introduced into the flocks 14 days after the second insemination. Day of conception was estimated retrospectively according to lambing dates (lambing date – 150 days).

Blood samples were collected before flushing (15th August), at the end of energy supplementation (6th September) and assayed for hormones and metabolites reflecting the energetic status (non-esterified fatty acid: NEFA; β-OH-butirate: BHB; insulin; insulin-like growth factor-I: IGF-I; thyroxin: T_4, 3,3',5-triiodothyronine: T_3) and plasma urea nitrogen (PUN; indicator for protein balance) of animals (Figure 1).

Body condition scoring

Condition was scored always by the same person using a 5 scale grade according to Thompson and Meyer (1994). This system is based on the level of muscling and fat deposition around the vertebral area, but did not take account of the energy stores of the fat-tail.

Laboratory procedures

Blood samples were collected in heparinized tubes and centrifuged within one hour. Plasma was separated, divided in 4 parts for the different hormone assays and stored at –20°C until processing.

Plasma IGF-I levels were assayed with a human extraction RIA kit (DSL-5600, Diagnostic System Laboratories, Inc., Webster, Texas) validated for sheep. Apart from the two control serum provided by the manufacturer, three different sheep plasma controls (low, medium, high) were used in duplicates to estimate intra- and inter-assay coefficients of variation (CV). (Intra- and inter-assay CV and sensitivity were 0.3–10.7%; 15% and 0.73–1.15 nmol/l, respectively.)

Plasma insulin levels were determined with a human Bi-Insulin IRMA kit (Cis Bio International, Gif-sur-Yvette, France) validated for sheep. Inter-assay coefficients ranged between 4.15–8.7%. The intra-assay coefficient was < 5%. Sensitivity ranged between 0.86–2.26 pmol/l.

The thyroid hormone levels were determined with ^{125}I-RIA. The T4 kit (125I-T4 CT-spec. RIA, Institute of Isotopes Co. Ltd., Budapest, Hungary) was originally developed for human use, and was slightly modified for assaying a lower concentration range of T_4 in animal samples (equine: Huszenicza et al., 2000; bovine: Meikle et al., 2004; ovine: Kulcsar et al., 2006). The inter- and intra-assay CV were determined by adding 3–5 parallels of low (mean: 10.5 nmol/l) and high (mean: 95.01 nmol/l) control plasma into each run of the assay. Depending on the concentrations the inter-assay CV varied between 8.5% and 3.2%, respectively. Intra-assay CV was 3.5–8.5% depending on the concentration of the control. Sensitivity was determined to be between 2.23 and 4.724 nmol/l.

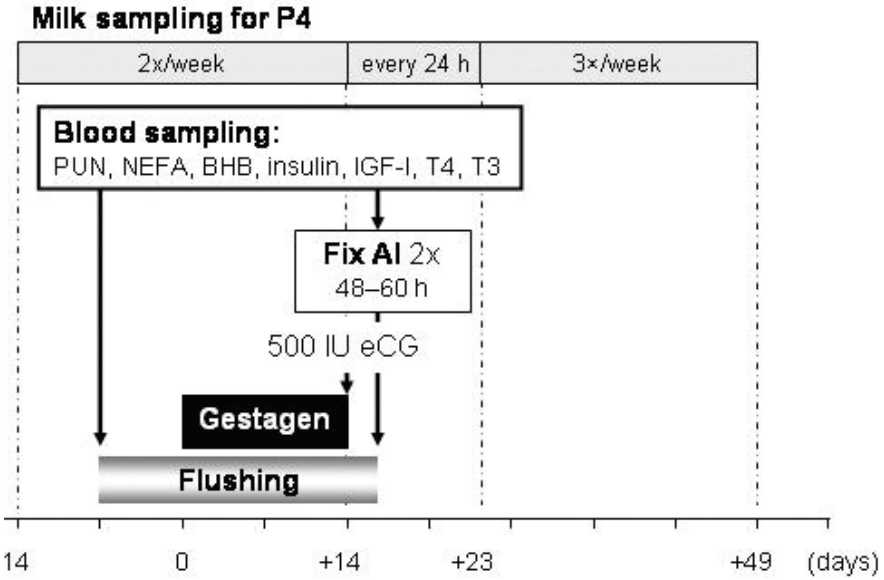

Figure 1. Experimental design

PUN = plasma urea nitrogen, NEFA = non-esterified fatty acid, BHB = β-hydroxi-butirate, IGF-I = insulin-like growth factor-I, T_4 = thyroxin, T_3 = triiodothyronin, eCG = equine serum gonadotropin, Fix AI = fix timed artificial insemination

The human T3 kit (125I-T3 CT RIA, Institute of Isotopes Co. Ltd., Budapest, Hungary) was suitable for assaying animal samples without modification in our earlier work (Huszenicza et al., 2000; Meikle et al., 2004; Kulcsar et al., 2006) (sensitivity: 0.326–1.196 nmol/l; inter-assay CV 3.89–20.7%; intra-assay CVs 7.8–13.0%).

Plasma NEFA concentrations were measured applying the colorimetric method (NEFA Reagent, Randox Laboratories Ltd., Ardmore, UK) as described by Nishina et al. (2003). BHB levels were determined by the use of a D-3-hydroxybutyrate kit (Randox Laboratories Ltd., Ardmore, UK). Plasma urea nitrogen was assayed with the use of a commercially available kit (Diagnosticum Ltd., Budapest).

Milk samples were collected in plastic tubes containing potassium-dichromate as preservative, and stored at 4°C until processing. The progesterone content of skimmed milk was determined with a locally developed microplate ELISA (Huszenicza et al., 1998; Nagy et al., 1998, Taponen et al., 2002), within 14 days of collection. According to our previous experiments in the same population, the threshold level for active *corpus luteum* was determined to be 4 nmol/l (data not shown). Samples were assayed as triplets and two different quality control samples were used. Interassay CVs were ≤ 20% and ≤ 16% for medium and high controls, respectively. The intraassay CV was < 10%. Sensitivity ranged between 0.53 ± 0.036 nmol/l (mean ± SEM).

Statistical analyses

Data are shown as mean ± the standard error of mean (SEM). Statistical analysis was performed using the R-statistical program (version R-2.2.1). To compare two means two-sided Student's *t*-test was applied. Comparison of proportions was estimated by the Chi-square test. Statistical significance was accepted at the 0.05 level.

RESULTS

Three ewes dried off before the end of the sampling period and were excluded from the final evaluation. The sampling protocol made possible the monitoring of ovarian activity before and during treatment, and to study ovarian response to the synchronization protocol. For further analysis of data, animals were grouped retrospectively according to their reproductive phase before the synchronization treatment (Figure 2). Among the 105 evaluated animals, those showing constantly low (< 4 nmol/l) milk progesterone levels before treatment were judged to be acyclic (n = 33). The other 72 dams which had at least one sample with elevated (> 4 nmol/l) progesterone levels before the gestagen insertion were found to have cyclic ovarian activity.

Among cyclic ewes 29 were found to be in the follicular phase by the day of gestagen insertion ($Cycl_F$; P_4 < 4.00 nmol/l on D 0), while an additional 43 ewes were in the luteal phase ($Cycl_{CL}$; P_4 > 4.00 nmol/l on D 0).

Among the 105 animals which presented with enough data for complete evaluation 26 conceived from fixed time AI, 72 became pregnant later from rams, and only seven failed to conceive at all. Almost half of the pregnant ewes (n = 98) delivered twins (n = 48=49%).

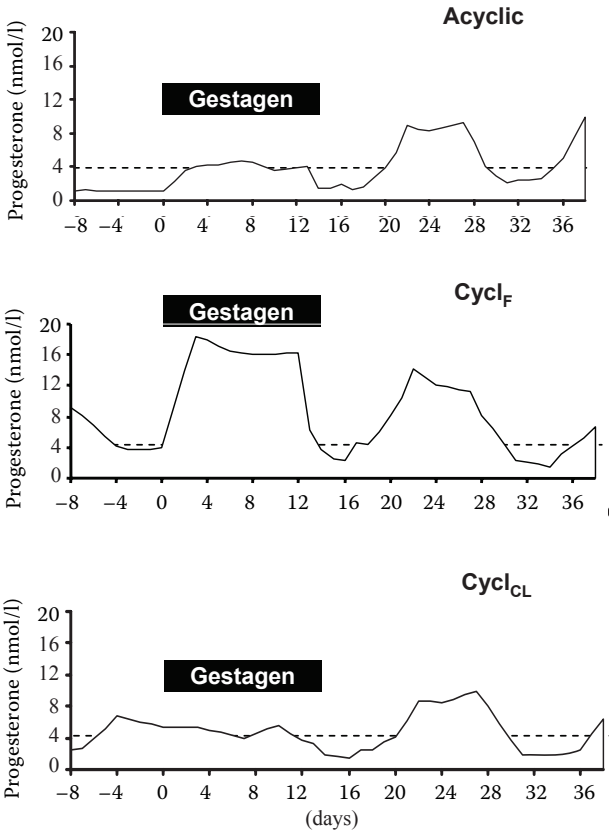

Figure 2. Typical progesterone profiles of ewes in different phases of cycle before gestagen treatment

$Cycl_F$ = follicular phase on the day of gestagen insertion, $Cycl_{CL}$ = luteal phase on the day of gestagen insertion

Ewes with cyclic ovaries before treatment continued to cycle following synchronisation, and 20 of them (28% of the previously cycling animals) conceived from fixed AI. Acyclic animals all became cyclic after treatment with the exception of one ewe. Six of the acyclic group (18%) conceived from the first estrous. Compared to the cycling groups acyclic ewes were younger and had lower BCS before flushing. However, the above mentioned difference in BCS was not reflected in body weight (BW). BCS and BW remained unchanged following flushing. Milk yield and the number of days in lactation did not differ between the groups (Table 2). NEFA and BHB levels in all groups remained within the physiological range during the whole experimental period (NEFA < 0.35 mmol/l; BHB < 0.80 mmol/l), with no signs of increased lipid mobilisation (data not shown). On the other hand acyclic animals had significantly lower insulin and IGF-I levels before flushing compared to cyclic groups. The flushing increased the plasma insulin level in both acyclic and cycling animals and before treatment differences became equalized. IGF-I levels followed the same pattern, but differences among groups were maintained after flushing (Table 3). T_3 and T_4 levels were slightly higher in cyclic groups compared to acyclic animals, but these differences were not significant. Thyroid hormone levels remained unchanged during flushing (data not shown).

Fix timed AI was successful in 20 of the cyclic ewes ($Cycl_F$: 8/29 = 28%, $Cycl_{CL}$: 12/43 = 28%; ns). Plasma urea nitrogen was significantly lower in animals which conceived from AI compared to non-pregnant ones (5.73 ± 0.24 vs. 6.36 ± 0.15 mmol/l; $P = 0.031$).

Table 2. Comparison of age, parity, lactation day, body condition score (BCS), body weight and milk yield of the acyclic and cyclic ewes at the beginning of gestagen treatment (mean ± SEM). *P*-values refer to two-sample *t*-test

	Ovarian function on Day 0 (beginning of gestagen treatment)		*P* =
	acyclic (*n* = 33)	cyclic (*n* = 72)	
Age (year)	3.56 ± 0.19	4.16 ± 0.19	0.026
Parity	2.7 ± 0.1	3.2 ± 0.2	0.042
Days from gestation to Day 0	154 ± 4	161 ± 3	0.177
Body condition score			
At the beginning of flushing	3.4 ± 0.1	3.9 ± 0.1	0.0001
At the end of flushing	3.5 ± 0.1	3.8 ± 0.1	0.046
Difference between them *P* =	0.474	0.227	
Body weight (kg)			
At the beginning of flushing	70.2 ± 0.1	73.1 ± 0.1	0.125
At the end of flushing	71.6 ± 0.1	74.6 ± 0.1	0.142
Difference between them *P* =	0.545	0.325	
Previous lactation			
Length of lactation (days)*	172 ± 13	202 ± 9	0.132
Milk yield (kg)*	226.7 ± 24.0	262.8 ± 19.1	0.317
Milk fat (kg)*	13.2 ± 1.3	15.4 ± 1.0	0.278
Milk protein (kg)*	11.1 ± 1.1 (*n* = 23 = 70%)	13.3 ± 0.9 (*n* = 59 = 82%)	0.204
Milk yield on Day 0			
Milk yield (kg/day)	1.48 ± 0.06	1.40 ± 0.03	0.296
Milk fat (g/day)	83.23 ± 6.17	81.70 ± 3.20	0.894
Milk protein (g/day)	69.66 ±2.47	68.90 ± 1.63	0.814

*on the ground of data of animals with at least 40 day long lactation

Table 3. Plasma insulin and IGF-I levels of acyclic and cyclic ewes at the beginning of the gestagen treatment (mean ± SEM). Two sample *t*-test

	Ovarian function on Day 0 (beginning of gestagen treatment)		*P* =
	acyclic (*n* = 33)	cyclic (*n* = 72)	
Insulin (pmol/l)			
At the beginning of flushing	5.30 ± 0.47	9.08 ± 0.51	0.0001
At the end of flushing	11.23 ± 0.89	11.33 ± 0.59	0.928
Difference between them *P* =	0.0001	0.006	
IGF-I (nmol/l)			
At the beginning of flushing	10.27 ± 0.35	13.16 ± 0.20	0.0001
At the end of flushing	13.16 ± 0.29	14.27 ± 0.22	0.0003
Difference between them *P* =	0.0001	0.0003	

The remainder of the ewes were impregnated by rams soon after insemination (Acyclic: 24/33 = 73%; $Cycl_F$: 19/29 = 66%; $Cycl_{CL}$: 27/43 = 63%; ns), only seven remained open (Acyclic: 3/33 = 9%; $Cycl_F$: 1/29 = 3%; $Cycl_{CL}$: 3/43 = 7%; ns) until the end of the sampling period. Twin lambing was slightly higher in $Cycl_{CL}$ dams compared to the other two groups (Acyclic: 12/30 = 40%; $Cycl_F$: 13/28 = 46%; $Cycl_{CL}$: 23/40 = 58%; ns).

According to P_4 profiles most of the Acyclic and $Cycl_F$ ewes ovulated within 24–48 hours after sponge insertion, and had active corpora lutea thereafter (Acyclic: 28/33 = 85%; $Cycl_F$: 27/29 = 93%; ns). Progesterone profiles did not indicate ovulation after Day 2–3 of gestagen treatment in any of the cases. At the time of gestagen removal altogether 76 ewes had active corpora lutea (Acyclic: 17/33 = 52%; $Cycl_F$: 27/29 = 93%; $Cycl_{CL}$: 32/43 = 74%; $P < 0.01$), indicating that in some case luteolysis occurred during gestagen treatment. The vast proportion of luteolysis during treatment was seen in those acyclic animals which ovulated at the beginning of the gestagen treatment (11/28 = 39%), and in those having active CL before sponge insertion ($Cycl_{CL}$: 9/43 = 21%). The above mentioned phenomenon did not occur in the $Cycl_F$ group (0/27%; $P < 0.001$). Ovulation at the beginning of gestagen treatment and luteolysis during treatment did not influence conception rates following timed AI (data not shown).

In some animals ($n = 10$) luteinisation of the dominant follicle present around the time of gestagen removal and eCG treatment was observed, and led

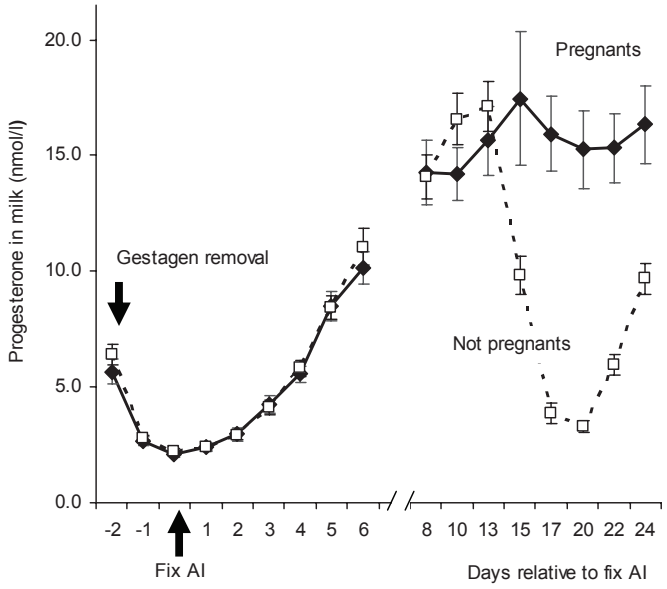

Figure 3. Intensity of luteinisation reflected by progesterone elevation in animals conceiving from fix timed AI compared to non-pregnant ewes (only among ewes giving normal ovarian response to synchronisation treatment; ♦ pregnant, □ non pregnant). From the day of insemination to Day 10, around the time of maternal recognition of conception (Day 12–16 following AI), around the day of the possible first rebreeding (average ± SEM). There are no significant differences over the first 13 days. In the following period $P = 0.015–0.0001$

to elevated P_4 levels at the time of insemination. This phenomenon was mostly seen in $Cycl_{CL}$ ewes (Acyclic: 1/33 =3%; $Cycl_F$: 2/29 = 7%; $Cycl_{CL}$: 7/43 = 16%; ns). In five cases the first luteal phase following synchronization was shorter than 10 days (sCL; Acyclic: 2/33 = 6%; $Cycl_F$: 0/29; $Cycl_{CL}$: 3/43 = 7%; ns). None of the above mentioned 15 dams conceived. The proportion of the animals with normal ovarian response following the synchronization protocol (*n* = 90 = 86%) was slightly lower in $Cycl_{CL}$ dams compared to Acyclic and $Cycl_F$ groups (Acyclic: 29/33 = 88%; $Cycl_F$: 27/29 = 93%; $Cycl_{CL}$: 34/43 = 79%; ns).

The intensity of luteinisation reflected by progesterone elevation in animals conceiving from fix timed AI compared to non-pregnant animals, among ewes exhibiting the normal ovarian response to synchronisation treatment, did not differ significantly over the first 13 days (Figure 3).

Animals conceiving from timed AI had significantly lower insulin levels before flushing compared to those which did not become pregnant (9.64 ± 0.84 vs. 7.36 ± 0.53 pmol/l; $P = 0.029$).

DISCUSSION

Energy-related metabolites and metabolic hormones

Awassi are fat-tailed sheep originating from the dry, subtropical zone of the Near East. Although seasonality in its homeland is not so strongly pronounced, it has a long breeding season with mating activity concentrated between late June and early September (Abu-Zanat et al., 2005; Tabbaa et al., 2008). According to our previous experiences with more westerly (Central European) populations reproductive activity becomes more seasonal with a shorter breeding season from the end of August until the end of December (data not shown). The synchronization treatment was performed at the beginning of the season.

In this experiment significantly higher insulin and IGF-I levels were found in ewes which were cycling before treatment compared to acyclic ones. Insulin and IGF-I in small ruminants number among those metabolic hormones which are involved in the signaling of actual energetic status and thus modulate central and peripheral regulatory mechanisms controlling the reproductive system. *In vitro* studies have shown insulin stimulated proliferation, steroid secretion capacity and morphologic differentiation of granulosa cells collected from cows (Spicer et al., 1993). *In vivo* experiments in dairy cows have shown that those nutrients which increase insulin secretion first enhance postpartum ovulation (Gong et al., 2002). In ewes energy supplementation (with lupin seed, glucose or amino-acid infusion) was shown to augment insulin levels, and at the same time enhance follicular growth and ovulation rate (Downing et al., 1995a,b,c). Some authors (Scaramuzzi et al., 2006) have hypothesized that insulin *per se* is not responsible for the above mentioned effects, but rather acts by mediating higher glucose intake at the ovarian level. This theory is supported by the detection of insulin-responsive glucose transporter molecules (GLUT-4) on the surface of granulosa and theca cells in ewes (Williams et al., 2001). In sheep granulosa cell cultures IGF-I has been shown to stimulate proliferation and differentiation of granulosa cells (Monniaux and Pisselet, 1992). In cows IGF-I has been demonstrated to increase the number of LH receptors (Spicer and Stewart, 1996) and gonadotrophin sensitivity of granulosa cells (Armstrong et al., 2001). *In vitro* findings concerning the positive effect of IGF-I on steroidogenesis of cow granulosa (Spicer et al., 1993) and theca cells (Spicer and Stewart, 1996) are consistent with clinical observations; in dairy cows dominant follicles of animals with higher IGF-I levels produce more β-estradiol and are more likely to ovulate from the first follicular wave compared to those having lower IGF-I levels (Beam and Butler, 1998). Although a small amount of IGF-I can be produced locally in the ovaries the main synthesis takes place in the liver under the regulation of growth hormones. IGF-I levels in the follicular fluid is highly correlated with plasma IGF-I levesl (Walters et al., 2002).

In the present study animals were at equal energetic statuses throughout the experimental period. Plasma metabolites did not indicate negative energetic balances or lipid mobilisation. At the same time the effect of flushing was demonstrated by the elevation of both insulin and IGF-I levels in all groups. In accordance with the literature animals with higher insulin and IGF-I levels were more likely to cycle than those showing lower levels.

Plasma urea nitrogen

Animals conceiving from timed AI had notably lower plasma urea nitrogen levels compared to

their flock-mates which did not conceive. In ruminants the plasma and milk urea concentration reflects protein supply. Therefore, by measuring urea nitrogen levels it is possible to follow up the effect of protein feeding and metabolism on reproductive performance (Butler, 1998). Several publications claim that in dairy cattle and ewes elevated crude protein in the ration leads to increased PUN levels which causes a decrease in conception rate (Butler et al., 1996; McEvoy et al., 1997; Rhoads et al., 2005). From the point of view of reproductive performance, PUN levels above 7 mmol/l are found to be critical. With elevated PUN concentration, uterine pH decreases (Elrod and Butler 1993; Rhoads et al., 2004), the ion concentration of the uterine fluid is altered, and prostaglandin secretion of *in vitro* endometrial cell cultures is also affected (Butler, 1998). These findings suggest that protein overfeeding during the first week of pregnancy leads to the alteration of the uterine environment, which may be detrimental for nidation and early embryo development.

Cycle stage before treatment and success of synchronization

Although some of the Acyclic and $Cycl_F$ animals ovulated on the first day of gestagen treatment, the success of fixed timed AI was similar in all groups. Regarding the presence or absence of a metabolically active *corpus luteum* on the day of gestagen removal, no difference was found in pregnancy rates following insemination. This is in accordance with previously published data in non-lactating goats, where Lassala et al. (2004) reported no difference in follicular dynamics and fertility between groups either with or without an active corpus luteum during estrus. On the other hand, in a certain proportion of animals the luteinisation of the dominant follicles may occur due to the eCG treatment used at the time of gestagen removal. Theis results in high progesterone levels at the time of insemination and these ewes have no real chance to conceive.

CONCLUSIONS

In the investigated population approximately one third of the ewes were still acyclic by the middle of August. We found significant endocrine differences between cycling and acyclic animals.

Elevated insulin and IGF-I levels were detected in animals which were cyclic before energy supplementation compared to acyclic ones. Higher circulating insulin and IGF-I levels could have beneficial effects at the ovarian level and may have stimulated folliculogenesis. Those animals which were acyclic before gestagen treatment or were in the follicular phase of the cycle may ovulate on the first day of gestagen treatment, but this did not influence the success rate of fixed timed AI. Similarly, it did not affect conception following insemination irrespective of whether luteolysis occurred during gestagen treatment or not, i.e., if a metabolically active corpus luteum was present or not on the ovary on the day of gestagen removal. On the contrary, it is certain that in a certain proportion of animals dominant follicles (a certain percentage) may luteinise due to the eCG treatment used at the time of gestagen removal. This results in high progesterone levels at the time of insemination and thus these ewes have no real chance of conceiving.

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Corresponding Author:

Aliz Marton, University of Pannonia, Georgikon Faculty, H-8360 Keszthely, Hungary
Tel. +36 83 545 370, Fax +36 83 510 167, E-mail: ma@georgikon.hu