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Induction of fertile cycles in the Blackhead sheep during the anoestrus period

Summary

A continuous supply of lambs is expected from the market, but not realizable without additional administration of hormones in seasonal breeds kept under extensive conditions. Our study had the aim to work out a possibility to induce fertile cycles in a flock of the Blackhead sheep already in early summer. Analyses of hormones progesterone and LH in untreated ewes were used, together with other tools, to control the different treatments for success, e.g. induction of ovulation, appearance of oestrus signs, regular luteal phases or pregnancy. Annual treatments were performed on altogether 76 ewes and started in June (1998), May (1999-2001), or April (2002). All tested variants had the main aim to overcome the GnRH/LH deficiency in the summer period. First series of experiments with either a single GnRH injection, a pheromone-containing paste or FSH showed unsatisfying results. An improvement was found after a previous progesterone priming which may have simulated a luteal phase of the oestrous cycle. Further modifications of the second part of treatment revealed a successful stimulation after as well a sequential GnRH agonist administration as a PMSG regime detected by plasma progesterone and ultrasonographic analyses. Finally, births of a single lamb and a pair of twins, respectively, already in October demonstrated the essential potency of both treatment regimes. However, a simplification, i.e. reducing the number of injections, and an optimisation of added hormone amounts are necessary. At the same time, changed conditions for keeping both the pregnant ewes and earlier born lambs have to be considered.

Key Words: sheep, anoestrous period, endocrine regulation, progesterone, GnRH, pregnancy

Zusammenfassung

Titel der Arbeit: **Auslösung fertiler Zyklen beim Schwarzköpfigen Fleischschaf im Anöstrus**

Vom Markt wird eine kontinuierliche Bereitstellung von Schlachtlämmern gewünscht. Dieses Ziel ist bei extensiv gehaltenen Rassen mit saisonalem Anöstrus nur durch zusätzliche Verabreichung von Hormonen zu erreichen. Wir untersuchten in einer Herde des Schwarzköpfigen Fleischschafes, die unter dem Gesichtspunkt „Landschaftspflege“ gehalten wurde, ob eine Hormonsubstitution in der Lage ist, bereits im frühen Sommer fertile Zyklen auszulösen (n = 76). Zu ausgewählten Zeitpunkten wurden Blutplasmakonzentrationen der Hormone Progesteron und LH bestimmt, um, zusammen mit weiteren Methoden, den Erfolg der Behandlungen, z.B. Auftreten von Östren, Auslösung von Ovulationen, von regulären Lutealphasen oder Vorliegen von Trächtigkeiten, zu kontrollieren. Die Behandlungen wurden einmal jährlich vorgenommen und im Juni (1998), Mai (1999-2001) oder April (2002) gestartet. Alle Varianten hatten das Ziel, den GnRH/LH-Mangel in der Sommerperiode auszugleichen. Die ersten Experimente mit einer einzelnen GnRH-Injektion, einer pheromon-haltigen Paste oder mit FSH blieben erfolglos. Eine Verbesserung wurde durch ein vorheriges Priming mit Progesteron zur Simulation einer Lutealphase erreicht. Von verschiedenen Modifikationen der Folgebehandlung waren sowohl durch eine sequentielle GnRH-Agonist - Administration als auch ein PMSG-Regime erfolgreiche Stimulationen möglich. Schließlich zeigten die bereits im Oktober erfolgten Geburten von einem Lamm bzw. einem Pärchen die grundsätzliche Eignung beider Kombinationen. Eine Vereinfachung der Behandlung, z.B. durch Reduzierung der Zahl der Injektionen und der Optimierung der zugeführten Hormonmengen, ist mit Sicherheit möglich. Parallel dazu sind in der zuchthygienischen Arbeit die veränderten Bedingungen für die Haltung sowohl der tragenden Muttern als auch der frühergeborenen Lämmer zu berücksichtigen.

Schlüsselwörter**:** Schaf, Anöstrus, endokrine Regulation, Progesteron, GnRH, Trächtigkeit

Introduction

During last years the main interest in sheep keeping raised the production of meat instead that of wool together with the increasing importance of sheep for the cultivation of landscape (PETERS, 2000; ZUPP and REHBOCK, 2000). At presence, the German sheep production enclosing nearly 2.8 millions of animals provides approximately 45 % of the German market (ZUPP, 2002). A growing interest arises to produce more and continuously lambs for the market. Such efforts are, however, limited for the pure-bred Blackhead and other meat breeds with a seasonal fertility whose percentage contributes actually approximately 90 % of the total stock. Begin and duration of season are breed, location and nutrition dependent and, moreover, affected by social signals from flockmates. Sheep and other domesticated species respond to the annual cycle in daylength to time the seasonal changes in food intake, fattening, gonadal activity, lactation and many other characteristics. Photoperiodic information is translated into a daily cycle of melatonin secretion (LINCOLN, 1992; ARENDT, 1998). Melatonin levels which are high at night and low during the day affect the pulsatile secretion of GnRH from the hypothalamus (MALPAUX et al., 1999) and, consequently, the LH release from the pituitary and presence or absence of ovulation. A complex management, the treatment by melatonin to simulate an autumn/winter-period and the stimulating effect of ram presence, was not successful to produce cyclic ewes in July or earlier, the time of year that is required to produce lambs for spring markets (O'CALLAGHAN, 1999). It was, therefore, the aim of this study in a flock of Blackhead ewes extensively kept for conservation of landscape evaluate other approaches to shorten the period between two breeding seasons. In cyclic ewes, the onset of the LH surge during the follicular phase is coincident with the initiation of a massive and sustained increase in GnRH secretion which continues well beyond the surge of LH and its amplitude may exceed that needed to generate the LH surge (BOWEN et al., 1998). The importance of prolonged GnRH secretion is seen in the maintenance of receptive behaviour, prolonging the initial triggering effect of estradiol (CARATY et al., 2002). It is known, that ovulation is blocked, when progesterone concentrations are elevated. However, progesterone priming seems to be essential for the full expression of the positive feedback effect of estradiol in inducing the preovulatory GnRH surge (CARATY and SKINNER, 1999). There is no seasonal difference in time course or amplitude of the LH surge as demonstrated in ovarectomized ewes which were held in an artificial luteal phase (MOENTER et al., 1990). The pituitary of acyclic ewes is fully equipped with GnRH receptor mRNA to respond to GnRH, but LH secretion and the rapid, transient increase in intracellular [Ca^{2+}] are uncoupled. The only limiting factor for LH secretion is, therefore, infrequent GnRH stimulation (GHOSH et al., 1996) compared with the situation in cyclic ewes (KARSCH et al., 1997). The hormonal imbalance results in failure of final follicular growth and ovulation in the non-breeding season. However, the total number of antral or ovulatory-sized follicles is comparable, and the largest follicles exhibit a wave-like growth pattern (SOUZA et al., 1996; REHBOCK et al., 1999; ABDENNEBI et al., 2002; GIUCCI and KAULFUSS, 2002). We have proofed the effectiveness of modified regimes of hormonal treatment which were used by others, e.g. BASIOUNI et al. (1996), KHALID et al. (1997), UNGERFELD and RUBIANES (1999), under our specific prerequisites. Our experiments in a flock of Blackheads were started after weaning in May of years 1998–2002 by administration of single

hormones followed later by combinations. The data of a previous three-years-study on the development of the plasma progesterone (P_4) concentration during anoestrus (REHBOCK et al., 1999) served as a basis for the evaluation of treatments. Effects of single or multiple injections of either a GnRH agonist, PMSG or FSH, given after a P_4 pretreatment were again recorded by the P_4 level, LH and FSH concentrations, oestrus signs, and ultrasonography.

Material and Methods

Animals and hormonal treatments

Annual experiments (Exp. I-V) were performed, beginning in 1997 and finished in 2002 (Table 1). During each year, Blackhead Mutton ewes (total n = 76) were randomly selected after lambing and weaning from the flock and kept either separately for the period of hormonal treatment and blood sampling, but mostly together with the flockmates on the pasture. Our efforts were directed on detection of significant changes in hormone levels detectable by a small number of samples. Bleeding was, therefore, restrained to preserve extensive conditions of keeping without needs for catheterisation. Ewes of Exp. III–V were held together with a fertile ram. The volume of blood samples taken from the vena jugularis by punctuation during short-time fixation was 6–10 ml which was drawn in commercial sampling tubes containing EDTA-K as anticoagulans (KABE, Nuembrecht, Germany) and centrifuged within 2h to separate plasma. Plasma aliquots were frozen at –20°C and held there until analysis of hormonal parameter. Following hormonal preparations were used in Exp. II–V (Table 1): GnRH agonist (Gonavet®; Veyx, Germany), a paste containing pheromone (friendly given by KAULFUSS), FSH (Ovagen™, The Netherlands), PMSG (eCG; Pregmagon®; IDT, Germany), and P_4 (P_4 valerate; Eifelfango, Germany), PGF_{2alpha} (Essex, Germany).

Table 1
Hormonal manipulations of anoestrous Blackhead Mutton sheep (n = 76) kept under extensive conditions throughout years 1997-2002 (Hormonelle Behandlungen anoestrischer SKF-Schafe (n = 76) unter Feldbedingungen während der Jahre 1997-2002)

Exp.	Year	Begin of treatment	n	Main elements of treatment	Data of 1st lambing after treatment
I	1997	March	6	no treatment (control)	January - March 1998
II	1998	June 24	16	50 µg GnRH agonist (GnRH) *or* 5 mL of a Pheromone-containing paste	January - March 1999
III	1999	May 14	12	1 x progesterone (P_4) + 4 x GnRH *or* insertion of a FSH releasing pump *or* 8 x FSH *or* 1x PMSG	February - March 2000
IV	2000	May 16	20	6 x P_4 + 4 x GnRH (each 250 µg) *or* 8 x FSH	February - March 2001
V	2001	May 15	20	5 x P_4 + 4 x GnRH (100 ng) + 1 x GnRH (50 µg) *or* 10 x P_4 + 1 x PMSG	October 16 (n = 2) 2001 *or* January – March
Va	2002	April 20	2	GnRH variant like 2001, but absence of a ram	

Exp. I was performed on untreated ewes (n = 6), in addition to a previous study (REHBOCK et al., 1999). It was done to characterize variation of steroid and LH levels in blood samples (n = 17) taken every 3 days during the anoestrous period, and some months later, during the spontaneous luteolysis within the breeding season. Exp. II had the aim to find out hormonal and clinical effects of either 5 ml of a pheromone containing paste to simulate an extended ram contact or a single injection by 50 µg of

GnRH agonist (each group n = 8). Blood sampling for P_4 analysis was continued every 2 to 3 days throughout d 30. Aim of Exp. III was to compare treatments by a) the combined stimulation of a single P_4 injection followed by 4 times 50 µg of GnRH (72, 75, 78, and 81 h later; n = 3), or b) 8 times FSH (8:00 and 14:00 on 4 consecutive days; n = 3), or c) implantation of an osmotic pump with a continuous release of FSH over 7 days (n = 3), or d) a single injection of PMSG (n = 3). A fertile ram was permanently present and a single blood P_4 analysis performed on d 28 was done to monitor luteal activity. Next, effects of a previous priming by six injections of P_4 followed by 4 injections of the GnRH agonist (each 50 µg; n = 10) were compared with those of an elevated blood gonadotropin level after multiple injections of FSH or its release from an osmotic pump (each n = 5) or a single administration of PMSG (n = 5; Exp. IV). Aim of Exp. V was to compare, besides the presence of a ram, the effects of either a combination of P_4 priming (5 injections) and GnRH agonist (G; n = 8) given in a modified manner as that used in Exp. IV or a combination priming (10 injections) followed by a single PMSG 24h later (P; n = 8). Four untreated ewes served as controls in studying the P_4 concentration. First group received 5 injections of Gonavet® 72, 75, 78, 81, 84 (in each case 100 ng) and 96 h (50 µg) after last P_4. Four animals left untreated. Bleeding was performed each experimental day at 8:00 throughout day 16 and additionally immediately before each injection of small dose of Gonavet® and 2 h after large peptide amount. Ultrasonography were used as a direct and P_4 analysis in blood samples as a indirect test for pregnancy on day 62. An identical regime of the combination priming and GnRH agonist, but in absence of a ram, was applicated in a repetition performed in 2002 (Exp. Va; n = 2).

Analytical methods

Changes affected by different hormonal treatments were studied by a combination of methods, i.e. the use of a fertile ram to mark females exhibiting oestrous signs and ultrasonography with the device SSD 500 (Aloka, Japan) equipped with 5.0 or 7.5 MHz probes to monitor the function of ovary and presence of a fetus, respectively. Specific and sensitive electrochemiluminescence-immunoassays applied for the gonadotropins LH (sandwich-type ECLIA) and FSH (competitive ECLIA), and [^{3}H]-RIA applied for the steroid hormones P_4 and estradiol-17β (E_2) were described elsewhere with all relevant characteristics including sensitivities and intra- and inter-assay variation (SCHNEIDER et al., 2002). Shortly, the non-radioactive LH assay uses a monoclonal antibody (mAB) against bovine LH (mAB 518 B7) which was labelled by a special ruthenium-compound according to the recommendation of IGEN (Gaithersburg, PA, USA), a standard preparation of bLH (Biotrend, Cologne, Germany), a polyclonal AB against bLH raised in rabbits (KANITZ et al., 1990), and a goat-anti-rabbit-AB coupled to magnetic beads (Dynal, Oslo, Norway) for the separation of bound and free fraction. The FSH assay was constructed by an ovine standard (oFSH; AFP 7571 A) which was labelled in a similar way as described for LH and a polyclonal AB against oFSH raised in rabbits (NIDDK-oFSH-1 AB), and the coupled second AB called already. Measurement of ECLIA methods was performed in the ORIGEN® 1.5-instrument (IGEN).

Statistical calculations

Sampling frequencies were a compromise between limitations by extensive conditions for sheep keeping and minimum needs to characterize specific reproductive processes. For each variant of the hormonal treatment, means ± S.D. were calculated. In this study, definitions for terms relating to gonadotropin concentrations are used as previously (SCHNEIDER et al., 2002): pulse – an increase in concentrations that lasts a short time (e.g. 10-60 min), basal concentrations – levels that underlie the pulses; surge – an increase in concentrations of long duration equivalent to the duration of many pulses.

Results

In Exp. I, mean concentrations of E_2, P_4 and LH during the early non-breeding period (March to May) reflected the depressed cyclic function, however, we found some individual differences. Low E_2 (data not shown) and P_4 levels measured in all samples of the first trial (Exp. I) indicated the absence of mature follicles and functional corpora lutea, respectively, in all 6 ewes, whereas the absolute height of mean LH was clearly influenced by individual 0–4 pulses during the sampling period (Fig. 1). In an

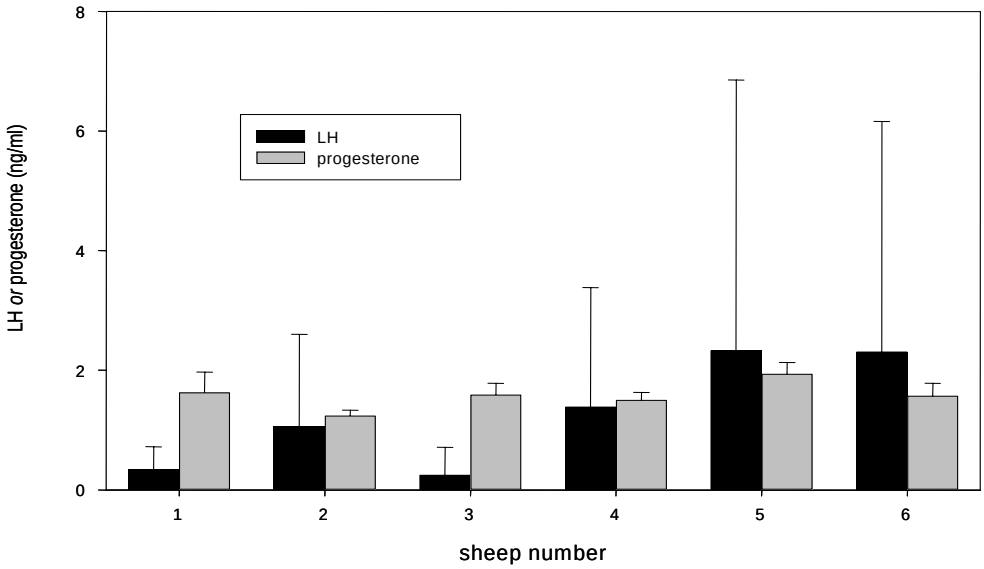

Fig. 1: Mean blood plasma concentrations of progesterone and LH (ng/ml) of Blackhead ewes (n = 6) during the early non-breeding season (March – May) (Mittlere Konzentrationen von Progesteron and LH im Blutplasma (ng/ml) von Schwarzköpfigen Fleischschafen (n = 6) während des frühen saisonalen Anöstrus (März – Mai))

additional sampling series throughout September/October, the preovulatory LH surge began approximately 58h after reaching basal P_4 concentrations and increased to more than 40 ng/ml plasma within 4 h (Fig. 2) which was 8 to 10 times higher than the amplitudes of the described pulses. A single blood sample from each animal taken on day 1 8:00 of Exp. II, before any treatment, showed low levels of P_4, LH and FSH (0.88 ± 0.12; 2.2 ± 2.6 and 3.20 ± 2.15 ng/ml, respectively; Fig. 3). The administration of 50 µg of Gonavet® induced a significant release of both gonadotropins within one hour which was higher for LH, but finished for both within 4 h after injection, whereas the P_4 level left unchanged over the sampling period. The application of the pheromone-containing paste did not influence the secretion of LH or FSH (data not

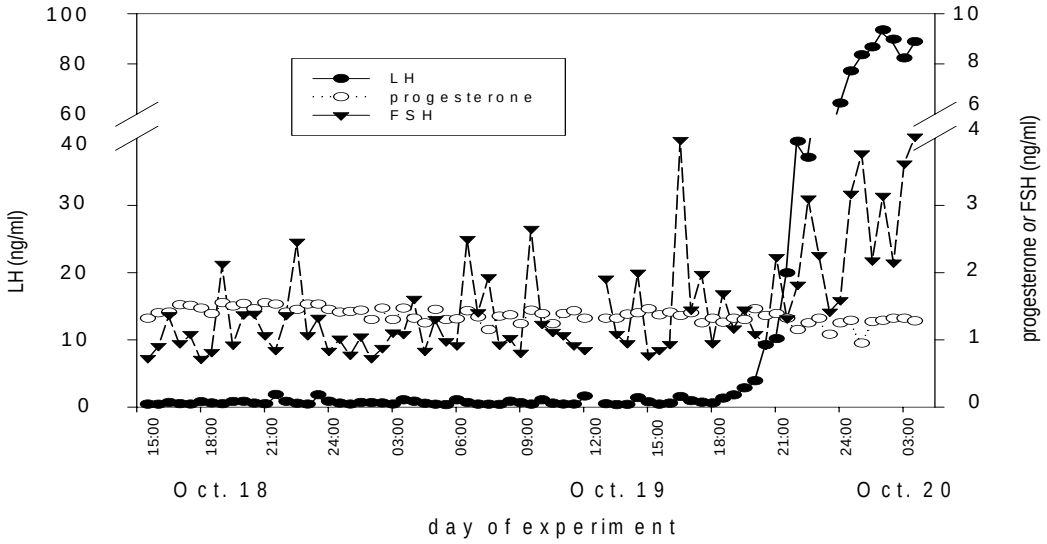

Fig. 2: Blood plasma concentrations of progesterone, LH and FSH (ng/ml) of a Blackhead ewe during the spontaneous luteolysis (Blutplasmakonzentrationen von Progesteron, LH und FSH (ng/ml) bei einem Schwarzköpfigen Fleischschaf während der spontanen Luteolyse)

shown). In 12 samples taken additionally from each ewe during further 4 weeks the P_4 concentration was lower than 1.2 ng/ml and indicating the absence of active corpora lutea.

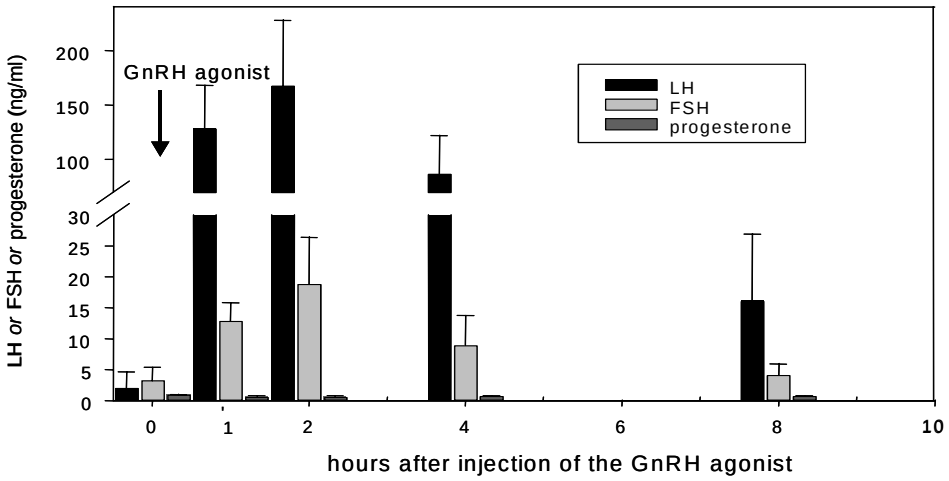

Fig. 3: Effects of a GnRH agonist on the release of progesterone, LH and FSH (ng/ml) of anoestrous Blackhead ewes (n = 8) (Beeinflussung der Freisetzung von Progesteron, LH und FSH (ng/ml) durch einen GnRH-Agonisten in anöstrischen Schwarzköpfigen Fleischschafen (n = 8))

Oestrus signs of different strength were observed both in FSH groups and in PMSG group, but not in the P_4-primed GnRH group (Exp. III). Priming resulted in an increase in blood P_4 from 0.67 ± 0.16 ng/ml before treatment to 1.53 ± 0.40 ng/ml 24h later, however, concentrations of next 2 days each analysed at 8:00 am (1.08 ± 0.52 and 0.89 ± 0.36 ng/ml) indicated the diminishing of P_4 from blood given only once. The P_4 level (Fig. 4) was low in all 8:00 samples of PMSG-treated animals, and only moderately elevated to 1.25 ± 0.33 ng/ml on day 6 in ewes treated by continuous FSH, but multiple FSH injections induced an increase in 2 from 3 animals between days 2, 5 and 7 (0.70 ± 0.21, 1.05 ± 0.18, and 3.01 ± 0.38 ng/ml, respectively) which could indicate previous ovulations. However, results from P_4 determinations performed during the

following period could not confirm the successful induction of luteal phases of normal length (data not shown).

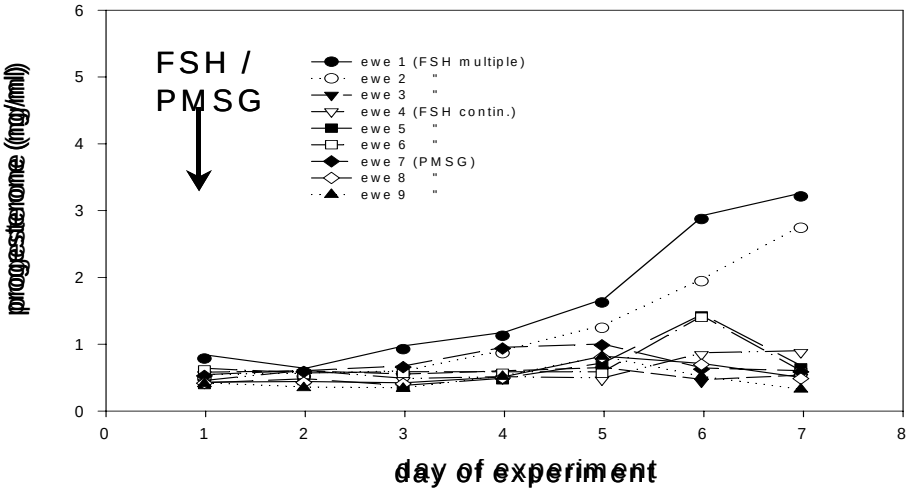

Fig. 4: Effect of exogenous FSH or PMSG on progesterone plasma levels (ng/ml) of Blackhead ewes (n = 9) during the non-breeding season (Beeinflussung der Progesteronkonzentration im Blutplasma (ng/ml) von Schwarzköpfigen Fleischschafen (n = 9) durch exogenes FSH oder PMSG)

In Exp. IV, the mean P_4 level of all ewes (n = 20) before any treatment was 0.95 ± 0.21 ng/ml. Treatment by exogenous P_4 and GnRH agonist induced various changes in blood levels of P_4, LH and FSH analysed in 5 ewes by repeated sampling (Table 2),

Table 2
Progesterone (P_4), LH and FSH plasma concentrations (ng/ml) in anoestrous ewes (n = 5) after treatment by exogenous P_4 and a GnRH agonist (GnRH; EXP. IV) (Progesteron (P_4), LH und FSH Plasmakonzentrationen (ng/ml) in anöstrischen Schafen (n = 5) nach Behandlung mit exogenem P_4 und einem GnRH-Agonist (GnRH); Exp. IV)

Day	Time	Measure	P_4		LH		FSH	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
1	08:00	P_4	1.03	0.22	0.96	0.23	1.30	0.33
2	08:00	P_4	1.72	0.24	1.64	0.41	1.91	0.41
3	08:00	P_4	2.31	0.96	1.53	0.36	1.35	0.29
	14:00		3.12	0.96	0.88	0.62	1.34	0.50
4	08:00	P_4	3.62	2.07	1.48	0.43	1.79	0.44
	14:00		3.34	0.43	0.76	0.55	1.59	0.48
5	08:00	P_4	2.42	0.23	1.16	0.58	1.57	0.39
	14:00		3.44	0.77	1.22	0.64	1.63	0.42
6	08:00	P_4	2.62	0.61	1.48	0.59	2.02	0.53
	14:00		3.57	0.63	1.57	0.47	1.95	0.24
7	08:00	GnRH	2.08	0.27	1.75	1.51	2.10	1.32
	09:00		2.02	0.38	34.32	0.33	3.40	1.66
	10:00		1.90	0.43	87.90	51.06	9.81	3.41
	11:00	GnRH	1.21	0.33	115.92	36.48	12.63	4.48
	12:00		1.96	0.40	76.14	34.93	9.44	5.39
	13:00		2.08	0.35	57.11	28.17	6.04	0.92
	14:00	GnRH	2.00	0.26	26.80	26.22	4.35	1.57
	15:00		1.89	0.38	22.93	12.41	4.05	1.86
	16:00		1.92	0.41	15.55	11.32	2.67	0.64
	17:00	GnRH	2.04	0.18	10.54	8.97	2.31	0.82
	18:00		1.88	0.27	7.04	5.76	1.87	1.04
	19:00		1.82	0.22	6.32	5.21	1.71	0.60
9	08:00		1.16	0.19	0.68	0.57	1.94	0.45

e.g. the P_4 concentration was elevated during the whole priming period and dropped moderately after the 6th injection of P_4 and 4 times GnRH agonist within 48 h to 1.16 ± 0.19 ng/ml. In contrary with this, the mean LH level was between 0.76 ± 0.55 and 1.75 ± 1.51 ng/ml in 8:00 and 14:00 samples from each day during priming, whereas the FSH level was in a comparable range (1.34 ± 0.50 and 2.10 ± 1.32 ng/ml, respectively). The 1st injection of GnRH agonist elevated both gonadotropin levels within some minutes, however, the increase in FSH was weaker than that of LH. Results showed that neither the 2nd nor the 3rd and the 4th injection of agonist were able to stimulate the further secretion of both hormones. Plasma concentrations reached nearly the pre-treatment levels at the end of sampling (Table 2). Endocrine results of the other treatment group demonstrated a steep increase in FSH after each of the eight injections of Ovagen™ by the factor 4 to 6, but the elevation was kept only for approximately 12h (data not shown). We found in 2 from 5 treated ewes an increase in plasma P_4 detectable already 24h after last injection and reaching 3.6 and 4.5 ng/ml, respectively, two days later. Further testation of plasma P_4 one and two weeks later resulted in basal levels like before treatment. Concentrations of P_4 were low in group G of Exp. V at begin of agonist administration but, as expected, not immediately before PMSG injection in another treatment group (0.93 ± 0.16 versus 1.64 ± 0.17 ng/ml; Fig.5).

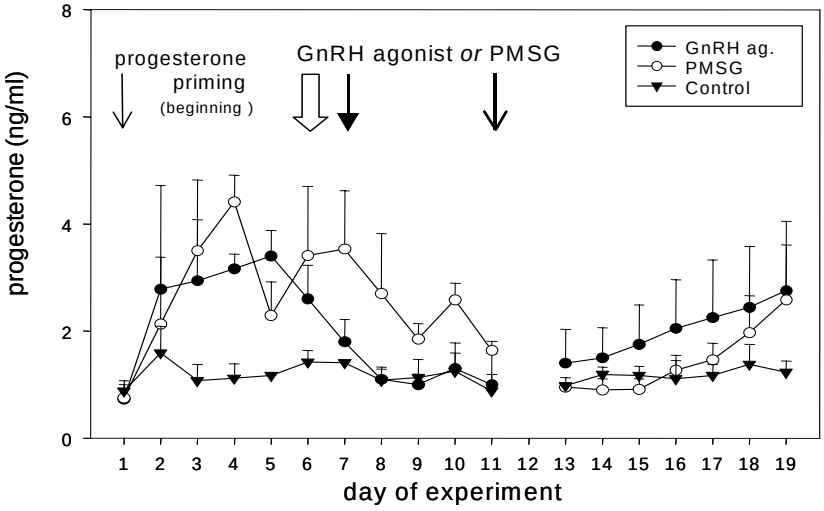

Fig. 5: Secretion of progesterone (ng/ml) during anoestrus after a previous progesterone priming and sequential treatment by a GnRH agonist (n = 8), single PMSG injection (n = 8), and in controls (n = 4), Exp. V (Progesteronsekretion (ng/ml) während des Anöstrus nach vorherigem Progesteron-Priming und sequentieller Behandlung mit einem GnRH-Agonisten (n = 8), einer einzelnen PMSG-Injektion (n=8) und in Kontrolltieren (n = 4), Exp. V)

As shown in Fig. 6, gonadotropin levels were not affected by first five injections of GnRH agonist (low dosage) but increased steeply after the 6th (high dosage). In both treatment groups, some ewes showed marks by the ram, however, there was a difference between GnRH agonist and PMSG treatment (2 from 8 versus 7 from 8 animals). Analysis of P_4 levels revealed an increase in 5 from 7 and 6 from 8, respectively, ewes throughout first after treatment-week (Fig. 5), whereas the steroid concentration left unchanged in controls (n = 4).

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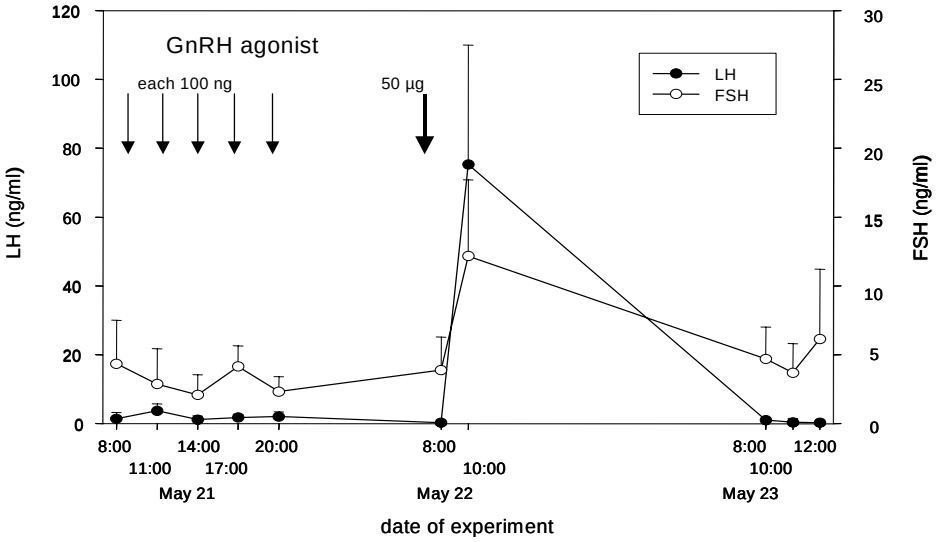

Fig. 6: Secretion of LH and FSH (ng/ml) of anoestrous ewes (n = 8) during the treatment period with a GnRH agonist (5 times 100 ng + once 50 µg) after previous priming (Sekretion von LH und FSH (ng/ml) in anöstrischen Schafen (n = 8) während der Behandlung mit einem GnRH-Agonist (5x 100 ng + 1x 50 µg) nach vorherigem Priming)

Two months after hormone substitution, animals from both treatment groups were found to be pregnant according to the result of ultrasonography (3 and 7, respectively, and further 2 ewes questionable; Fig. 7).

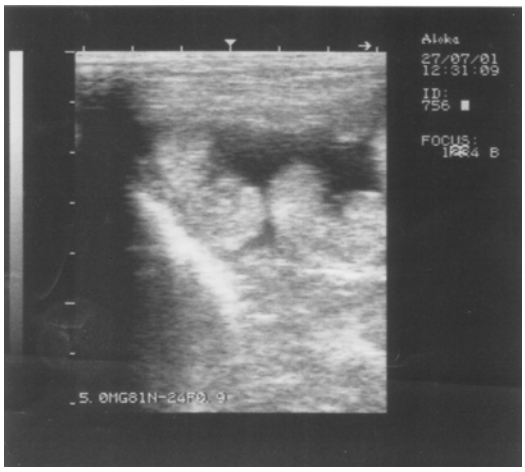

Fig. 7: Ultrasonographic detection of twins in a Blackhead ewe on day 62 of pregnancy after combined treatment with priming and PMSG during anoestrus (Ultraschallnachweis von Zwillingen bei einem Schwarzköpfigen Fleischschaf am 62. Trächtigkeitstag nach kombinierter Behandlung mit Priming and PMSG während des Anöstrus)

Elevated P_4 concentrations in blood samples taken at the same time confirmed this result indicating the presence of an active corpus luteum in 3 animals (> 3.0 ng/ml) and were questionable in another 4 (1.1 - 1.6 ng/ml). Ewes were spent to the stall one month before the expected parturition. A male lamb was born after priming and GnRH agonist treatment, and a pair of twins after priming and PMSG (Fig. 8).

Fig. 8: Three months old lambs (single + twins) with their mothers born in October after hormonal treatment during anoestrus (Drei Monate alte Lämmer (Einzeltier und Zwillingspärchen) mit ihren Müttern, geboren im Oktober nach hormoneller Behandlung im Anöstrus)

The identical treatment of 2 further ewes by the combination priming and sequential GnRH agonist one year later (Exp. Va), but in absence of a ram, confirmed the results of previous experiments, e.g. comparable levels of gonadotropins (Fig. 9) and P_4 which were lower than 2 ng/ml before treatment, increased after exogenous P_4 to 3 - 5 ng/ml, and luteal phases with a length of 7 - 9 days after previous ovulations.

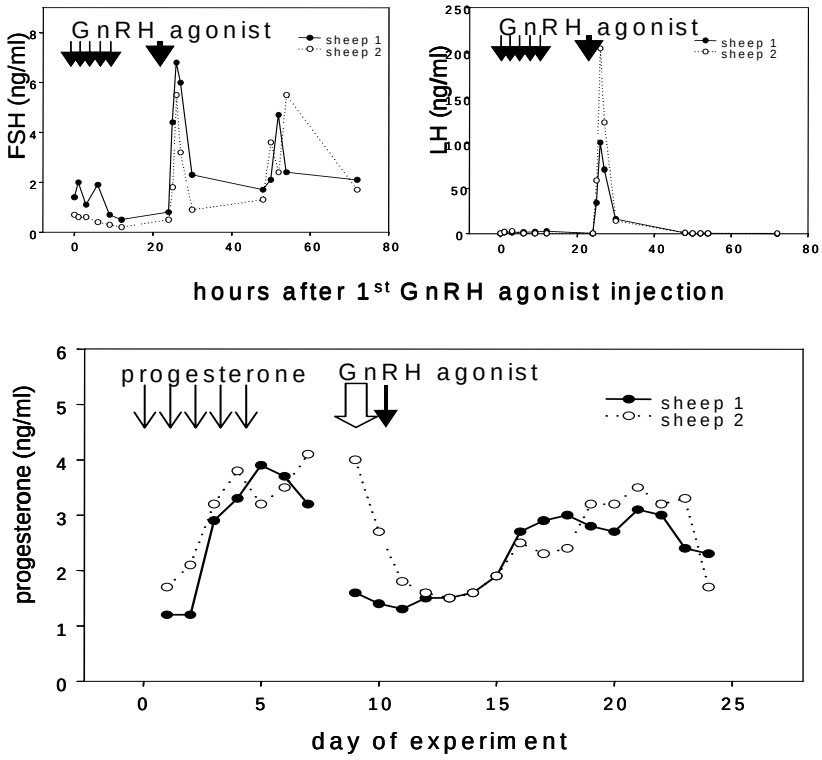

Fig. 9: Individual LH, FSH and progesterone secretion (ng/ml) of Blackhead ewes (n = 2) during the sequential treatment with the GnRH agonist in the early non-breeding period (April) (Individuelle LH-, FSH- und Progesteron-Sekretion (ng/ml) von Schwarzköpfigen Fleischschafen (n = 2) während der sequentiellen Behandlung mit dem GnRH-Agonist im frühen Anöstrus (April))

In the sum, our experiments showed that only a combination of P_4 priming and exogenous GnRH/gonadotropin may suitable to advance the begin of the breeding season in the Blackhead by some months. The reason is seen in the need of an endocrine situation which is similar under all relevant aspects with that of the oestrous cycle.

Discussion

A continuous production of lambs nearly over the year is only possible in the Blackhead sheep like other breeds with a seasonal reproductive function by a combination of different measures like intensive selection and introduction of a ram to the flock. Pheromones stored in the wool, wax and urine of ram are effective to increase ovarian activity (KAULFUSS et al., 1997; REKWOT et al., 2001; ZUNIGA et al., 2001), however, it seems to be impossible to obtain fertile cycles without an additional hormonal treatment under conditions of the extensive sheep keeping, e.g. for cultivation of landscape. It was, therefore, the aim of our study to develop a complex treatment regime that considers as well the main endocrine interrelationships during seasonal and nonseasonal period as practical regulations. Cyclic oestrus behaviour usually appears at the end of summer or the beginning of autumn and finishes in winter or at the very beginning of spring (GALLEGOS-SANCHEZ et al., 1998). Considering a 5months-duration of pregnancy in sheep the lambing period is concentrated in months December to February. There are breed-depending differences and, moreover, other factors exist that may influence time of year when ewes are sexually active or inactive. Although both small and medium-sized follicles and low levels of peripheral plasma P_4 levels have been detected (KAULFUSS et al., 1997; REHBOCK et al., 1999; BARTLEWSKI et al., 2000), a limitation of ovarian activity which is characterized by the absence of pre-ovulatory follicles with normal secretory rate is observed (SOUZA et al., 1996). The reason can be seen in day-light affected changes of the secretion of melatonin which amplifies the negative feedback response of the hypothalamus to ovarian P_4. An infrequent GnRH and LH pulse rate of 1 per 6 - 12h in anoestrus results compared with 1 per 30 - 60min in the follicular phase of the oestrous cycle (GALLEGOS-SANCHEZ et al., 1998; O'CALLAGHAN, 1999). The only limiting factor for LH secretion is, therefore, infrequent GnRH stimulation (GHOSH et al., 1996) compared with the situation in cyclic ewes (KARSCH et al., 1997). Moreover, there is a changed LH reception by ovarian cells because differences have been observed in the occurrence of splice variants of mRNA encoding the LH receptor between follicles taken throughout season or the anoestrous period (ABDENNEBI et al., 2002), whereas all the forms of the bovine LH receptor mRNA increased in a coordinated manner during the development of cyclic corpus luteum (KAWATE and OKUDA, 1998). The simplest variant to overcome the anoestrous period by exogenous hormones seems to mimic a short-day period of year by melatonin application. Experimental data from the literature showed that exogenous melatonin give results that show a high degree of variation related to breed, management system, and environmental and physiological factors. A melatonin treatment may have, therefore, a restrained potential to shorten considerably the anoestrous period and, parallel with that, improve reproductive performance (HARESIGN, 1992; BRUNET et al., 1995; ZUNIGA et al., 2002). Long-term results of melatonin application have yet to be fully evaluated, but a refractoriness of

treatment can not excluded, because animals seem to need a period of long days (ARENDT, 1998). Following the route of melatonin effect across endogenous opiate peptides (EOP) of brain, further possibilities may arise from an intervention in that mechanism. Really, it was found that administration of naloxone, an EOP antagonist, revealed an earlier onset of oestrus, increased length and earlier LH surge in anoestrous ewes hormonally treated for the induction of cycles (FUENTES et al., 2001). Next, it is well-known that administration of GnRH or one of its potent agonists induces an immediate LH release, however, the resulting surge should have an appropriate shape like the endogenous GnRH maximum. In cyclic ewes, the onset of the LH surge during the follicular phase is coincident with the initiation of a massive and sustained increase in GnRH secretion which continues well beyond the surge of LH and its amplitude may exceed that needed to generate the LH surge (BOWEN et al., 1998). The importance of prolonged GnRH secretion is seen in the maintenance of receptive behaviour, prolonging the initial triggering effect of P_4 (CARATY et al., 2002). Blockade of GnRH action, e.g. by a GnRH antagonist, terminates the further release of LH (EVANS et al., 1996). An inadequate treatment with exogenous GnRH may, therefore, unfavourably influence the number and quality of pituitary GnRH receptors (SCHNEIDER et al., 2002) or ovarian LH receptors (ABDENNEBI et al., 2002), the occurrence of oestrus signs or the corpus luteum development. Thus, GnRH given in a pulsatile manner, e.g. every 2h for 24 - 48h in the non-breeding period induced the LH surge and ovulation, but these effects were often followed with a shortened luteal phase (BASIOUNI et al., 1996). Unsatisfying results of numerous studies demonstrate the complexity of the neuroendocrine regulation of reproductive processes, thus, it seems to be important to begin each treatment out-of-season with a P_4 priming, i.e. a pre-treatment with P_4 or synthetic progestagens (DANIEL et al., 2001). Aims of this step are to mimic the luteal phase of the oestrous cycle and the existence of the negative feedback of P_4 which is responsible for the inhibition of LH pulse frequency (GOODMAN et al., 1995) and to intensify oestrus signs. There are large differences in the length and dose of P_4 administration and the distance to further treatments that should enable the development of the positive E_2 feedback on hypothalamus and pituitary that results from a decrease in the response of the pituitary to GnRH and an inhibition of the GnRH pulse size (EVANS et al., 1994; GOODMAN et al., 1995). Elevated endogenous P_4 may directly interfere with the activation of E_2-responsive neural systems to block both surges (RICHTER et al., 2002), although the E_2 signal is relatively short (7 - 14h) and may only effective during the pre-surge period. A limitation of the usefulness of E_2 measurements in sheep under practical conditions can be derived. On the other hand, EVANS et al. (1996) have found that development and progression of the preovulatory LH surge in sheep depend upon GnRH stimulation throughout its entire time course. In literature, priming was performed by different ways, e.g. a single P_4 injection before a GnRH treatment was not able to eliminate defective luteal function through early stages of follicle development (BASIOUNI et al., 1996; KHALID et al., 1997). A short-term priming, i.e. a treatment over a period of 3 – 6 days (KNIGHTS et al. 2001) has the advantage of allowing more flexibility in length of priming and more flexibility in treatment protocols under field conditions (UNGERFELD and RUBIANES, 1999). Going-on in the route of melatonin action, priming and exogenous gonadotropins of different origin were used by numerous researchers (e.g. UNGERFELD and RUBIANES, 1999;

KNIGHTS et al., 2001; STENBAK et al., 2001). Results by others groups demonstrated he usefulness of different combinations of hormones to overcome the blockade of ovarian function in anoestrous ewes. In our study which was tightly accompanied by a complex of control measures, healthy lambs were born in time in both treatment groups. This result has demonstrated that a moderate stimulation of anoestrous ewes by exogenous hormones is able to advance the begin of the breeding season thus significantly extending the period for lamb production. Further studies, however, are necessary to optimise the hormonal treatment and to combine it with measures of keeping and breeding.

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References

ABDENNEBI, L.; LESPORT, A.S.; REMY, J.J.; GREBERT, D.; PISSELET, C.; MONNIAUX, D.; SALESSE, R.:
Differences in splicing of mRNA encoding LH receptor in theca cells according to breeding season in ewes. Reproduction **123** (2002), 819-825

ARENDT, J.:
Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Reviews of Reproduction **3** (1998), 13-22

BARTLEWSKI, P.M.; VANDERPOL, J.; BEARD, A.P.; COOK, S.J.; RAWLINGS, N.C.:
Ovarian antral follicular dynamics and their associations with peripheral concentrations of gonadotropins and ovarian steroids in anoestrous Finnish Landrace ewes. Anim. Reprod. Sci. **58** (2000), 273-291

BASIOUNI, G.F.; KHALID, M.; HARESIGN, W.:
Effect of bovine follicular fluid treatment and progesterone priming on luteal function in GnRH-treated seasonally anoestrous ewes. Anim. Sci. **62** (1996), 443-450

BOWEN, J.M.; DAHL, G.E.; EVANS, N.P.; THRUN, L.A.; WANG, Y.; BROWN, M.B.; KARSCH, F.J.:
Importance of the gonadotropin-releasing hormone (GnRH) surge for the induction of the preovulatory luteinizing hormone surge of the ewe: dose-response relationship and excess of GnRH. Endocrinology **139** (1998), 588-595

BRUNET, A.G.; LOPEZ SEBASTIAN, A.; PICAZO, R.A.; CABELLOS, B.; GODDARD, S.:
Reproductive response and LH secretion in ewes treated with melatonin implants and induced to ovulate with the ram effect. Anim. Reprod. Sci. **39** (1995), 23-34

CARATY, A.; SKINNER, D.C.:
Progesterone priming is essential for the full expression of the positive feedback effect of estradiol in inducing the preovulatory gonadotropin-releasing hormone surge in the ewe. Endocrinology **140** (1999), 165-170

CARATY, A.; DELALEU, B.; CHESNEAU, D.; FABRE-NYS, C.:
Sequential role of E2 and GnRH for the expression of estrous behavior in ewes. Endocrinology **143** (2002), 139-145

DANIEL, J.A.; STERLE, S.W.; McFADIN-BUFF, E.L.; KEISLER, D.H.:
Breeding ewes out-of-season using melengestrol acetate, one injection of progesterone, or a controlled internal drug releasing device. Theriogenology **56** (2001), 105-110

EVANS, N.P.; DAHL, G.E.; GLOVER, B.H.; KARSCH, F.J.:
Central regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion by estradiol during the period leading up to the preovulatory GnRH surge in the ewe. Endocrinology **134** (1994), 1806-1811

EVANS, N.P.; DAHL, G.E.; CARATY, A.; PADMANABHAN, V.; THRUN, L.A.; KARSCH, F.J.:
How much of the gonadotropin-releasing hormone (GnRH) surge is required for generation of the luteinizing hormone surge in the ewe? Duration of the endogenous GnRH signal. Endocrinology **137** (1996), 4730-4737

FUENTES, V.O.; SANCHEZ, V.; ROSILES, R.; FUENTES, P.I.:
The effect of low doses of naloxone on the preovulatory surge of LH and on the onset and duration of estrus in the ewe with induced oestrus during the non-breeding season. Anim. Reprod. Sci. **65** (2001), 225-230

GALLEGOS-SANCHEZ, J.; MALPAUX, P.; THIERY, J.C.:
Control of pulsatile LH secretion during seasonal anoestrus in the ewe. Reprod. Nutr. Dev. **38** (1998), 3-15

GHOSH, B.R.; WU, J.C.; MILLER, C.L.:
Gonadotropin-releasing hormone-stimulated calcium mobilization is altered in pituitary cultures from anestrous ewes. Biol. Reprod. **54** (1996), 753-760

GOODMAN, R.L.; PARFITT, D.B.; EVANS, N.P.; DAHL, G.E.; KARSCH, F.J.:
Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. Endocrinology **136** (1995), 2412-2420

GIUCCI, E.; KAULFUSS, K.H.:
A comparing study using ultrasonography to determine the seasonality of the reproduction in sheep. 35. Jahrestagung Physiologie und Pathologie der Fortpflanzung, 14.02.-15.02.2002, Leipzig, P2.10

HARESIGN, W.:
Manipulation of reproduction in sheep. J. Reprod. Fertil. Suppl. **45** (1992), 127-139

KANITZ, E.; KANITZ, W.; DELZER, W.; SCHMIDT, H.E.; KITZIG, M.; SCHNEIDER, F.:
Die Nutzung der LH-Analytik zur Bestimmung des optimalen Besamungszeitraumes nach Superovulationsbehandlungen. Mh. Vet. Med. **45** (1990), 123-125

KARSCH, F.J.; BOWEN, J.M.; CARATY, A.; EVANS, N.P.; MOENTER, S.M.:
Gonadotropin-releasing hormone requirements for ovulation. Biol. Reprod. **56** (1997), 303-309

KAULFUSS, K.H.; SUESS, R.; RUMMER, K.; PRANGE, H.; BORELL, E. von:
Ovarian reaction after pheromone application in anoestrous German Mutton Merino ewes in relation to ovary state before stimulation. EAAP - 48th Annual Meeting, Vienna 1997, Book of Abstracts No. 3, p. 289, S 3.6

KAWATE, N.; OKUDA, K.:
Coordinated expression of splice variants for luteinizing hormone receptor messenger RNA during the development of bovine corpora lutea. Molec. Reprod. Develop. **51** (1998), 66-75

KHALID, M.; BASIOUNI, G.F.; HARESIGN, W.:
Effect of progesterone pre-treatment on steroid secretion rates and follicular fluid insulin-like growth factor-1 concentrations in seasonally anoestrous ewes treated with gonadotrophin releasing hormone. Anim. Reprod. Sci. **46** (1997), 69-78

KNIGHTS, M.; MAZE, T.D.; BRIDGES, P.J.; LEWIS, P.E.; INSKEEP, E.K.:
Short-term treatment with a controlled internal drug releasing (CIDR) device and FSH to induce fertile estrus and increase prolificacy in anestrous ewes. Theriogenology **55** (2001), 1181-1191

LINCOLN, G.A.:
Photoperiod-pineal-hypothalamic relay in sheep. Anim. Reprod. Sci. **28** (1992), 203-217

MALPAUX, B.; THIERY, J.C.; CHEMINEAU, P.:
Melatonin and the seasonal control of reproduction. Reprod. Nutr. Dev. **39** (1999), 355-366

MOENTER, S.M.; CARATY, A.; KARSCH, F.J.:
The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology **127** (1990), 1375-1384

O'CALLAGHAN, D.:
A practical approach to the management of reproductive seasonality in sheep. Reprod. Dom. Anim. **34** (1999), 285-291

PETERS, K.J.:
Zuechterische Aspekte der Lammfleischproduktion. Arch. Tierz., Dummerstorf **43** (2000) Sonderheft, 98-105

REHBOCK, F.; SCHNEIDER, F.; ZUPP, W.; GRUMBACH, S.:
Seasonal influences on blood plasma progesterone concentration in ewes of Blackhead Mutton sheep breed (short communication). Arch. Tierz., Dummerstorf **42** (1999), 183-189

REKWOT, P.I.; OGWU, D.; OYEDIPE, E.O.; SEKONI, V.O.:
The role of pheromones and biostimulation in animal reproduction. Anim. Reprod. Sci. **65** (2001), 157-170

RICHTER, T.A.; ROBINSON, J.E.; EVANS, N.P.:
Progesterone blocks the estradiol-stimulated luteinizing hormone surge by disrupting activation in response to a stimulatory estradiol signal in the ewe. Biol. Reprod. **67** (2002), 119-125

ROSA, H.J.D.; JUNIPER, D.T.; BRYANT, M.J.:
Effects of recent sexual experience and melatonin treatment of rams on plasma testosterone concentration, sexual behaviour and ability to induce ovulation in seasonally anoestrous ewes. J. Reprod. Fertil. **120** (2000), 169-176

SCHNEIDER, F.; BELLMANN, A.; BECKER, F.; BAMBANG PUERNOMO, S.; REHFELDT, C.; NUERNBERG, G.; KANITZ, W.:
Gonadotropin release in periovulatory heifers after GnRH analogs measured by two types of immunoassays. Exp. Clin. Endocrinol. Diabetes **110** (2002), 1-10

SOUZA, C.J.H.; CAMPBELL, B.K.; BAIRD, D.T.:
Follicular dynamics and ovarian steroid secretion in sheep during anoestrus. J. Reprod. Fertil. **108** (1996), 101-106

STENBAK, T.K.; REDMER, D.A.; BERGINSKI, H.R.; ERICKSON, H.S.; NAWANUKRAW, C.; TOUTGES, M.J.; BILSKI, J.J.; KIRSCH, J.D.; KRAFT, K.C.; REYNOLDS, L.P.; GRAZUL-BILSKA, A.T.:
Effects of follicle stimulating hormone (FSH) on follicular development, oocyte retrieval, and in vitro fertilization (IVF) in ewes during breeding season and seasonal anestrus. Theriogenology **56** (2001), 51-64

UNGERFELD, R.; RUBIANES, E.:
Effectiveness of short-term progestogen primings for the induction of fertile oestrus with eCG in ewes during late seasonal anoestrus. Anim. Sci. **68** (1999), 349-353

ZUNIGA, O.; FORCADA, F.; ABECIA, J.A.:
The effect of melatonin implants on the response to the male effect and on the subsequent cyclicity of Rasa Aragonesa ewes implanted in April. Theriogenology **72** (2002), 165-174

ZUPP, W.:
In: Agrarprofi – Landesforschungsanstalt fuer Landwirtschaft und Fischerei M-V, AgriMedia Verlag, 2002, 05.06.03-04

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Wie saisonal sind Fleischschafe? Bauernzeitung (2000) 18, 66-67

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