doi: 10.17221/7982-VETMED

Determination of the proper time for mating after oestrous synchronisation during anoestrous or oestrous by measuring electrical resistance of cervical mucus in ewes

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ABSTRACT: The purpose of the present study was to investigate whether the electrical resistance values of the cervical mucus and/or blood oestradiol-17β and progesterone concentrations at mating, after oestrous synchronisation during the anoestrous or oestrous period in ewes, are related to fertility. Oestrus was induced by medroxyprogesterone acetate-impregnated intravaginal sponges (Veramix, Upjohn, USA), either for 12 days (12-day group; 24 Kymi and 28 Chios) or for 14 days (14-day group; 24 Kymi and 28 Chios), plus 400 IU equine chorionic gonadotropin (Intergonan, Intervet, the Netherlands) *i.m.*, at the time of sponge removal. In each case, one blood sample was collected from all ewes at sponge insertion, at sponge removal and at oestrus, for the determination of serum oestradiol-17β and progesterone concentrations, using a radioimmunoassay. The electrical resistance of the cervical mucus was measured once, just after oestrus detection by teaser rams, using a digital heat detector. All ewes found to be in oestrus were mated to fertile rams. Pregnancy was determined by transabdominal ultrasonography 35–40 days after mating. Kymi ewes were in anoestrous, while those of Chios were in the oestrous period. The 14-day treatment resulted in better outcome as concerns oestrus detection and pregnancy rate than the 12-day treatment, in either oestrous or anoestrous period ewes. Oestradiol-17β concentrations at sponge removal and at oestrus were lower ($P < 0.05$) in the 14-day group than in the 12-day group, in both oestrous or anoestrous period ewes. Progesterone concentrations at sponge insertion and removal were higher ($P < 0.05$), while progesterone concentrations and electrical resistance values of the cervical mucus at oestrus were lower ($P < 0.05$) in ewes of both groups who conceived compared to those that did not, either in oestrous or in the anoestrous period. Linear regression analysis revealed a positive relation between the electrical resistance values of the cervical mucus and blood serum progesterone concentrations in both oestrous or anoestrous period ewes. The electrical resistance of cervical mucus could be useful for the detection of the proper time for mating after oestrous synchronisation in ewes.

Keywords: electrical resistance of cervical mucus; oestradiol-17β; progesterone; anoestrous period; oestrous period; ewes

List of abbreviations

eCG = equine chorionic gonadotropin, ERCM = electrical resistance of cervical mucus, MAP = medroxyprogesterone acetate

Economic pressures on ewe producers act as a stimulus for the improvement of reproductive efficiency. Accurate oestrus detection is of paramount importance for successful mating and for sustaining high pregnancy rates after oestrous synchronisation. Unfortunately, this represents a major problem for ewe producers because it is time-consuming, demands personnel and leads to extra costs. Thus, many ewe producers avoid oestrus detection and leave rams in the ewe flock, in order to increase the pregnancy rate during the oestrous period. However, such an approach severely

doi: 10.17221/7982-VETMED

hampers the gathering of accurate data regarding breeding. Furthermore, a reluctance of rams with respect to oestrus detection is observed during the anoestrous period. If there was a means to detect oestrus by other methods (apart from rams), ewe producers could use rams only for mating and could also keep accurate data.

Bioelectrical impedance measurement of vaginal mucosa, near the cervix uteri, is a useful tool for oestrus detection, ovulation and appropriate time of mating or insemination (Gupta and Purohit 2001; Rorie et al. 2002; Yamauchi et al. 2009). It is well known that vaginal resistance throughout the oestrous cycle decreases gradually in the follicular phase and reaches the lowest value in pigs (Rezac et al. 2002), and sheep (Bartlewski et al. 1999), during pro-oestrus, while in cattle (Aboul-Ela et al. 1983), goats (Rezac et al. 2001), buffaloes (Gupta and Purohit 2001), and horses (Brook 1982), the lowest resistance is during oestrus. Alterations in cervical mucus and, therefore, resistance are also known to be under the influence of oestrogens and progesterone (Rezac 2008). In some cases, merely a change in total impedance contains enough information for correlation with the biological event (Valentimuzzi et al. 1996). Rorie et al. (2002) noted that repeated measurement of intravaginal electrical resistance demands extra labour; thus, this method for oestrus detection would be more applicable to animals after oestrous synchronisation. When used correctly, electrical resistance probes are effective in identifying females in oestrus. Thus, vaginal resistance could be a very viable alternative, if enough data are available (Gupta and Purohit 2001). Frequent observation for oestrous activity could be reduced or eliminated, and animals with limited or a lack of oestrous signs could be mated at the appropriate time (Yamauchi et al. 2009). Thus, the purpose of the present study was to determine the suitability of electrical resistance of the cervical mucus (ERCM) as an alternative index, alone or in combination with blood ovarian steroids, for accurate oestrus detection in ewes in the anoestrous or oestrous period.

MATERIAL AND METHODS

Animals and oestrous synchronisation. Two commonly used protocols for oestrous synchronisation were used in two indigenous breeds of ewes (Kymi and Chios). There is a very short anoestrous period in a large proportion of Chios ewes (Bosniakou 1983; Avdi et al. 1993), and Chios ewes were therefore studied during the oestrous period, while Kymi ewes were studied during the anoestrous period.

The study was conducted at the N.AG.RE.F. – Agricultural Research Station of Halkidiki, in northern Greece, in April when Kymi ewes are in anoestrous (Tsiligianni 2014) and Chios ewes are still in the oestrous period (Bosniakou 1983). Kymi (n = 48) and Chios (n = 56) ewes, aged from 30 to 42 months and weighing 43.00 ± 1.30 kg (Kymi) and 65.00 ± 1.50 kg (Chios) were kept in a sheltered barn, and had access to a natural pasture and water *ad libitum*. The ewes were in their second or third lactation period and had their last lambing two to three months before sponge insertion. Oestrus was induced by MAP-impregnated intravaginal sponges (60 mg Veramix, Upjohn, USA), either for 12 days (12-day group; 24 Kymi and 28 Chios ewes) or for 14 days (14-day group; 24 Kymi and 28 Chios ewes), plus 400 IU eCG (Intergonan, Intervet, the Netherlands) *i.m.*, at the time of sponge removal. Oestrus detection was carried out using two teaser rams, every 12 h for 72 h, starting 12 h after sponge removal. Any ewe accepting to be mounted by the ram was considered as being in oestrus. Eight rams of known proven fertility (four Kymi and four Chios) were used for mating, just after oestrus detection. All ewes were removed from the ram just after mating.

Blood sampling. One blood sample was collected from each ewe by jugular venipuncture (Venoject, Terumo, Belgium) at sponge insertion, at sponge removal and at oestrus (after oestrus detection by teaser rams and before the natural mating). After clotting, blood samples were centrifuged (1100 × g; 20 min; 4 °C) and serum was aspirated and stored at – 20 °C until assayed.

Oestradiol-17β and progesterone assays. Oestradiol-17β and progesterone concentrations in blood serum were determined using a radioimmunoassay (RIA) after extraction as described by Martin et al. (1987), with minor modifications (Rekkas et al. 1991). The radiolabelled oestradiol and progesterone were provided by Amersham Biotech (Buckinghamshire, UK). Oestradiol-17β and progesterone antiserums were developed by the Institute of Molecular Biology, Iraklion, Crete, Greece (Theodosiadou et al. 2004). The lower limit

doi: 10.17221/7982-VETMED

of sensitivity was 3.90 pg/ml and 0.019 ng/ml, the intra-assay variability was 2.2–3.4% and 1.8–2.8% (n = 6), the inter assay variability was 8.5% and 7.4% (n = 18) and the recovery rate was 92.3% ± 2.4% and 94.5% ± 2.2% (mean ± SD; n = 18) for oestradiol-17β and progesterone, respectively.

Electrical resistance of the cervical mucus (ERCM). ERCM has been extensively studied in cows and sows; however, data for ewes are rather scarce. In the present study, only one measurement of ERCM was taken, just after oestrus detection by teaser rams and before the natural mating, using a digital oestrus detector for cows (Cyclus, A.S. Lima, Sandnes, Norway), as previously described (Theodosiadou et al. 2014), in order to determine the proper time for mating in ewes.

Pregnancy diagnosis. Pregnancy diagnosis was carried out by trans-abdominal ultrasonography (SonoVet 2000; 4.5 to 6 MHz convex transducer; MedisonCO, Seoul, Korea) on days 35 to 40 after mating.

Statistical analysis. Pearson's chi-square test was used to compare the oestrus detection rate (%) or the pregnancy rate (%) between the 12-day and the 14-day group, as well as between Kymi (anoestrous period) and Chios (oestrous period) ewes. Student's t-test (independent) was used to compare oestradiol-17β concentrations, progesterone concentrations or ERCM values between the 12-day and the 14-day group, between Kymi (anoestrous period) and Chios (oestrous period) ewes, as well as between ewes that conceived and those that did not. The results are expressed as means ± SEM. Linear regression analysis was performed to determine any possible relationship between ERCM values and oestradiol-17β or progesterone concentrations in the blood serum of Kymi (anoestrous period) or Chios (oestrous period) ewes. Statistical analysis was performed using SPSS® 15.0 for Windows (SPSS Inc., Athens, A.E., Greece). A probability of $P < 0.05$ was the minimum level of significance, in all cases.

RESULTS

Oestrus detection rate (%) and pregnancy rate (%) were significantly lower in the 12-day group compared to the 14-day group, in both Kymi (anoestrous period) and Chios (oestrous period) ewes (Table 1). In the 12-day group, five Kymi and six Chios ewes were found to be in oestrus on the 1st day, six Kymi and seven Chios ewes on the 2nd day, while the rest of the ewes were found to be in oestrus on the 3rd day of detection. In the 14-day group, eight Kymi and seven Chios ewes were found to be in oestrus on the 1st day, 10 Kymi and 12 Chios ewes were in oestrus on the 2nd day, while the rest of the ewes were in oestrus on the 3rd day of detection.

The electrical resistance values (ohms) of the cervical mucus (ERCM values) at oestrus were significantly lower in ewes that conceived compared to those that did not, in both the 12-day and 14-day group and in both Kymi (anoestrous period) and Chios (oestrous period) ewes (Table 1). Blood serum oestradiol-17β concentrations (pg/ml) and progesterone concentrations (ng/ml) in the 12-day and 14-day groups, in Kymi (anoestrous period) and Chios (oestrous period) ewes (those that conceived and those which did not), at all-time points studied (mean ± SEM) are presented in Table 2.

Significant differences concerning oestradiol-17β and progesterone concentrations were observed

Table 1. Oestrus detection rate (%), pregnancy rate (%) and electrical resistance values (ohms) of the cervical mucus (ERCM, mean ± SEM) at oestrus, in Kymi (anoestrous period) ewes (n = 48) and Chios (oestrous period) ewes (n = 56) that received sponges for 12 days (12-day group) or 14 days (14-day group)

	Kymi ewes (anoestrous period)		Chios ewes (oestrous period)	
	12-day group	14-day group	12-day group	14-day group
Oestrus detection rate (%)	58.33^a	100.00^b	50.00^a	92.86^b
Pregnancy rate (%)	41.67^a	83.33^b	42.86^a	85.71^b
ERCM values (ohms)				
Conceived	362.45 ± 31.52*	276.20 ± 28.77*	267.50 ± 16.09*	297.83 ± 23.06*
Non-conceived	805.00 ± 106.81	558.57 ± 67.42	652.50 ± 99.85	595.00 ± 60.62

^{a,b}significant differences between 12-day group and 14-day group, in each breed ($P < 0.05$)

*significant differences between conceived and non-conceived ewes, in each group and in each breed ($P < 0.05$)

doi: 10.17221/7982-VETMED

Table 2. Oestradiol-17β (pg/ml) and progesterone (ng/ml) concentrations in blood serum of Kymi (anoestrous period) (n = 48) and Chios (oestrous period) (n = 56) conceived or non-conceived ewes receiving sponges for 12 days (12-day group) or 14 days (14-day group), at different time points (mean ± SEM)

	Oestradiol-17β concentration (pg/ml)				Progesterone concentration (ng/ml)			
	Kymi ewes (anoestrous period)		Chios ewes (oestrous period)		Kymi ewes (anoestrous period)		Chios ewes (oestrous period)	
	12-day group	14-day group	12-day group	14-day group	12-day group	14-day group	12-day group	14-day group
Sponges insertion								
Conceived	84.72 ± 6.54	95.22 ± 5.44	91.56 ± 6.86	88.92 ± 4.52	0.21 ± 0.02^{ax}	1.02 ± 0.11^{bx}	1.95 ± 0.25^{y}	1.58 ± 0.22^{y}
Non-conceived	98.58 ± 4.59	98.11 ± 0.71	94.71 ± 5.04	86.98 ± 6.25	0.07 ± 0.009^{ax*}	0.09 ± 0.004^{bx*}	0.99 ± 0.16^{y*}	0.79 ± 0.06^{y*}
Sponges removal								
Conceived	42.12 ± 3.11^{ax}	28.93 ± 2.20^{bx}	68.07 ± 4.36^{ay}	46.76 ± 2.53^{by}	0.14 ± 0.01^{ax}	0.20 ± 0.01^{bx}	0.42 ± 0.06^{ay}	0.56 ± 0.04^{by}
Non-conceived	38.45 ± 3.04^{ax}	22.16 ± 3.26^{bx}	66.03 ± 2.68^{ay}	53.25 ± 4.30^{by}	0.08 ± 0.006^{ax*}	0.11 ± 0.02^{bx*}	0.15 ± 0.02^{ay*}	0.25 ± 0.04^{by*}
Oestrus								
Conceived	34.83 ± 2.65^{a}	23.73 ± 2.65^{b}	43.06 ± 3.07^{a}	21.47 ± 2.55^{b}	0.12 ± 0.02^{x}	0.12 ± 0.01^{x}	0.29 ± 0.05^{y}	0.40 ± 0.03^{y}
Non-conceived	31.38 ± 3.47^{a}	18.06 ± 0.79^{b}	30.65 ± 5.51^{a}	17.82 ± 1.81^{b}	0.32 ± 0.03^{x*}	0.41 ± 0.08^{x*}	0.57 ± 0.06^{y*}	0.76 ± 0.09^{y*}

^{a,b}significant differences between 12-day group and 14-day group, in each breed (P < 0.05)
^{x,y}significant differences between Kymi and Chios ewes, in each group (P < 0.05)
*significant differences between conceived and non-conceived ewes, in each group and in each breed (P < 0.05)

between ewes that conceived and those that did not, between the 12-day group and the 14-day group and between Kymi (anoestrous period) and Chios (oestrous period) ewes, at the different time points studied (Table 2).

Finally, in oestrus, linear regression analysis revealed a significant positive relation between the ERCM values (ohms) and blood serum progesterone concentrations (ng/ml), in Kymi (anoestrous period) ewes (ERCM = 1115.119x + 212.751, x = progesterone concentration in ng/ml; R^2 = 0.456, P < 0.05, n = 48) and Chios (oestrous period) ewes (ERCM = 917.516x + 1.011, x = progesterone concentration in ng/ml; R^2 = 0.562, P < 0.05, n = 56).

DISCUSSION

Successful mating of ewes relies on efficient and accurate oestrus detection. Visual observation after the introduction of "teaser" (apron-fitted or vasectomised) rams remains the only available method for oestrus detection in ewes; all the commercially available electronic devices that could improve the efficiency of oestrus detection and increase the pregnancy rate are adapted only for cows and gilts (Canfield and Butler 1989; Yamauchi et al. 2009). Monitoring changes in vaginal resistance could be a reliable alternative to visual observation of oestrus in ewes. It is well known that vaginal resistance changes throughout the oestrous cycle and its lowest values occur around oestrus. With respect to these considerations, the aim of the present study was to test a method available for cows and sows for its efficiency and accuracy in detecting oestrus in ewes.

In the present study, all Kymi ewes were at the full anoestrous period (Tsiligianni 2014), according to progesterone concentrations (< 1.0 ng/ml) at the time of sponge insertion (Menegatos et al. 1995). Kymi ewes that received sponges for 14 days had higher progesterone concentrations than those that received sponges for 12 days. This might indicate that Kymi ewes with higher progesterone concentrations could be closer to the oestrous period. Both groups of Chios ewes had higher progesterone concentrations at sponge insertion than the corresponding groups of Kymi ewes, indicating that Chios ewes were in the oestrous period (Bosniakou 1983), taking into consideration the month (April) in which this study was conducted.

doi: 10.17221/7982-VETMED

Oestradiol-17β concentrations at sponge removal and at oestrus were higher in ewes that received sponges for 12 days compared with ewes that received sponges for 14 days, in both Kymi (anoestrous period) and Chios (oestrous period) ewes. Furthermore, in both the oestrous and anoestrous period lower oestrus detection and pregnancy rates were observed when ewes received sponges for 12 days. This difference in oestradiol-17β concentrations could be related to the fact that administration of sponges for 14 days was more effective with respect to oestrus detection and pregnancy rate. It has been suggested that the diameter of ovarian follicles is a very important factor for successful ovulation (Kohno et al. 2005); furthermore, the largest antral follicles are the main source of oestradiol and there are periods of increased oestradiol secretion throughout the oestrous cycle in ewes (Bartlewski et al. 1999; Duggavathi et al. 2006). The higher oestradiol-17β concentrations at oestrus, when sponges remain *in situ* for 12 days may be related to the late ovulation and lower pregnancy rate in this group, since follicle diameters could be linked with oestradiol-17β concentrations and pregnancy rates.

Progesterone concentrations at sponge insertion and removal were higher in ewes that conceived compared to those that did not, in both groups and in both Kymi (anoestrous period) and Chios (oestrous period) ewes; thus, progesterone levels appear to be important for conception. With regard to sponge removal progesterone concentrations were higher in both Chios groups (oestrous period) compared to Kymi ewes (anoestrous period), while levels were higher in ewes of both breeds that had received sponges for 14 days. Low progesterone levels during the luteal phase related to the permanence of aged follicles in their static or early atretic phase, and their ovulation was reported to result in low fertility in ewes (Ungerfeld and Rubianes 1999). It is possible that follicles growing in a wave in all phases of the ovine oestrous cycle may prepare for luteinisation by producing progesterone (Duggavathi et al. 2006). It is suggested that the diameter of follicles may be affected by progesterone or that progesterone concentrations could be related to the diameter of follicles and ovulation. Thus, progesterone levels at sponge insertion and/or sponge removal may be linked with follicle diameter, ovulation and pregnancy rates.

At oestrus, progesterone concentrations in both groups and in all ewes that conceived (anoestrous or oestrous period) were lower compared to those that did not. Previous studies in ewes showed that progesterone concentrations are lower at the beginning of synchronised oestrus and start to increase towards the end of oestrus (Kouskoura et al. 1995; Theodosiadou et al. 2004). Taking the above into account, ewes that conceived might be mated before the LH surge and at the beginning of oestrus, whilst those that did not conceive may be mated late in oestrus. In addition, changes in progesterone concentrations in the genital tract could influence sperm capacitation (Barboni et al. 1995), acrosome reaction (Cheng et al. 1998) and probably sperm motility and chemoattraction. Thus, with respect to all these observations, the increased progesterone concentrations in the ewes that did not conceive might have negatively affected spermatozoa (capacitation or acrosome reaction), directly or indirectly via alterations in ERCM.

In the present study, only one measurement of ERCM was taken, just after the detection of oestrus by teaser rams and before the natural mating, in order to determine the proper time for mating. We chose to measure ERCM just after oestrus detection by teaser rams, because mating is usually performed at this time point. Electrical resistance of cervical mucus was < 300 ohms in the 12-day group in the oestrous period (Chios ewes) and both 14-day groups [oestrous (Chios ewes) and anoestrous (Kymi ewes) period] and < 400 ohms in the 12-day group in the anoestrous period (Kymi) in ewes that conceived. Furthermore, it was lower in ewes that conceived compared to those that did not both during the oestrous and during the anoestrous period. These values could be the cut-off point for mating. Furthermore, the ERCM just before cervical artificial insemination (AI) or mating was significantly lower in ewes that conceived compared to those that did not irrespective of whether AI was performed at a fixed time or after oestrus detection by teaser rams (Theodosiadou et al. 2014) and regardless of if all ewes were detected in oestrus by teaser rams just before mating (Tsiligianni 2014).

At oestrus a significant positive relationship was revealed between the ERCM values and blood serum progesterone concentrations, in both the Kymi (anoestrous period) and Chios (oestrous period) ewes studied. Similar results have been reported in cattle (McCaughey and Patterson 1981), ewes (Bartlewski et al. 1999), and buffaloes (Gupta and Purohit 2001). On the other hand, in our study and

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in accordance with previous studies (Bartlewski et al. 1998), no relationship between ERCM and serum oestradiol-17β concentrations at oestrus was observed. These findings suggest that ERCM is probably influenced by alterations in circulating levels of progesterone during oestrus. In ewes, cervical mucus volume, crystallisation and spinnbarkeit increase after oestrous synchronisation by MAP (Stefanakis 1988); these differences result from changes in hormonal patterns and could be reflected in the ERCM. A close association between the lowest value of vaginal impedance during the follicular phase of the oestrous cycle and manifestations of oestrous behaviour was observed in many species (Rezac 2008). Furthermore, follicular development is accompanied by an elevation in plasma oestradiol levels (Menegatos et al. 2003; Ginther et al. 2005), which induce female receptivity and may be linked to the decrease in vaginal impedance. Thus, ERCM could also provide useful information about the development of preovulatory antral follicles in ewes.

CONCLUSIONS

In conclusion, oestrus detection and pregnancy rates were significantly higher when sponges remained *in situ* for 14 days, in ewes either in the oestrous (Chios) or anoestrous (Kymi) period. Serum progesterone concentrations at sponge insertion and removal, as well as at oestrus were a critical parameter with respect to whether the ewe conceived or not. Low electrical resistance values of the cervical mucus at the time of mating could be a valuable indicator for prediction of conception in ewes. However, further research is needed to determine the best time for the ERCM measurement.

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Received: 2014–05–14
Accepted after corrections: 2015–01–06

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