

Induced changes on uterine myoelectrical activity around oestrous in the ewe by progesterone antagonist Onapristone and superovulation treatments – preliminary results

Alterações na actividade mioeléctrica uterina na ovelha induzidas durante o estro por tratamentos de superovulação e pelo antagonista da progesterona Onapristone – resultados preliminares

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Summary: Uterine myoelectrical activity (EMG) was measured with a computerised modular system in one adult Merino ewe previously fitted with three pairs of electrodes embedded in the myometrium of the uterine body (UB), the medium horn (MH) and proximal horn (PH).

EMG was recorded for 30 minutes periods during follicular (F) and luteal (L) phases of normal oestrus cycle, and 24 and 28 hours after sponge withdrawal in synchronized and eCG superovulated oestrus cycles associated (treatment SZK) or not (treatment S) with the progesterone antagonist Onapristone.

To evaluate uterine myoelectrical total activity the bursts of spike potentials (events) taken in each 30 minutes record were counted. The number of events in each uterine portion was expressed as a percentage of the total activity in each treatment (F, L, S and SZK). Frequency represents the number of events recorded over a 10 minute period.

F and L treatments exhibited different patterns of EMG, with F presenting a significant higher activity on the posterior portions of genital tract (UB), and L presenting higher activity on the anterior portions (PH and MH). Pattern of uterine myoelectrical activity during normal follicular phase (treatment F) was modified both by eCG (S) and Onapristone (SZK) treatments. In both treatments it was observed not only an increase on frequency, but also a different pattern of percentage of total activity, with PH presenting the highest percentage of total activity on both treatments.

In this work important changes on both quality and quantity of ewe uterine myoelectrical activity were found, associated with eCG and Onapristone. Although needing further confirmation on a larger number of animals, these changes may explain the reduction or absence of fertilization previously associated with superovulatory protocols and Onapristone administration.

Resumo: A actividade mioeléctrica uterina (EMG) foi medida, utilizando um sistema modular computadorizado, numa ovelha adulta com 3 pares de eléctrodos previamente implantados no miométrio da região do corpo uterino (UB), do corno médio (MH) e do corno proximal (PH).

Foram realizados registos de EMG com a duração de 30 minutos durante as fases folicular (F) e lútea (L) de um ciclo éstrico

normal, e ainda 24 e 48 horas pós-remoção das esponjas, em ciclos éstricos superovulados com eCG associada (tratamento SZK) ou não (tratamento S) ao antagonista da progesterona Onapristone. A análise da actividade mioeléctrica uterina total foi realizada através da contagem de picos de actividade registados em cada sessão de 30 minutos. O número de picos em cada porção uterina foi expresso como uma percentagem da actividade mioeléctrica uterina total. O número de picos de actividade contados em 10 minutos representa a frequência.

Os tratamentos F e L apresentaram diferentes padrões de EMG. Em F foi registada maior actividade na porção posterior do tracto uterino (UB), enquanto em L foi registada maior actividade nas porções anteriores (PH e MH). O padrão de actividade mioeléctrica uterina obtido durante a fase folicular normal (tratamento F) foi modificado pelo tratamento superovulatório isolado (tratamento S) e associado ao antagonista da progesterona (tratamento SZK). Nestes tratamentos registou-se não só um aumento da frequência, como também um padrão diferente da percentagem de actividade total, sendo PH a porção uterina com maior percentagem de actividade total em ambos os tratamentos.

Neste trabalho foram identificadas alterações na actividade mioeléctrica uterina da ovelha, associadas ao eCG e ao Onapristone, que apesar de necessitarem confirmação num maior número de animais, poderão justificar a redução ou ausência de fertilização associadas aos regimes superovulatórios e à administração de Onapristone.

Introduction

Superovulatory protocols are still limited by the large variation in results obtained, and reduction on fertility rates are frequently observed probably due to modifications on sperm transport (Whyman and Moore, 1980; Evans and Armstrong, 1984; Hawk et al., 1987). In a previous work (Cavaco Gonçalves et al., 1997) an absence of fertilization was observed after Onapristone administration during the preovulatory period of superovulated ewes, although better oestrus synchronization was obtained; no effects were observed on ovulation or oocyte maturation.

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Uterine motility is closely dependent on the propagation of myometrial action potentials. The myometrium is a target organ for steroid hormones (Bouftila and Clabaut, 2001; Langendijk et al., 2005). Thus, fluctuations of progesterone and 17β -oestradiol ratio observed during oestrous cycle are responsible for the variations of uterine motility pattern, being the high levels of 17β -oestradiol and the low levels of progesterone observed around oestrous responsible for the increase of uterine motility detected at that time.

In this work uterine myoelectrical activity (EMG) was evaluated in sheep during the follicular (F) and luteal (L) phases of normal oestrous cycle and compared with EMG obtained during follicular phase after superovulation with eCG, associated or not with the progesterone antagonist Onapristone.

Materials and methods

The experiment was carried out in one adult Merino ewe housed in an individual pen, weighing 61.5 kg and exposed to conditions of natural daylight. The ewe was fed with hay supplemented with commercial pelleted food, and water ad libitum. The animal was previously fitted with 3 pairs of monopolar teflon electrodes (Amplifon, Amplaid Division, Milano, Italy) through a laparotomy performed under general anaesthesia. An atraumatic needle was fixed at one of the extremities of the electrodes and about 1-2 mm of the electrodes coating was removed near that end. The electrodes within each pair were positioned 1-2 mm apart, with the bared area embedded in the muscle layers of the uterine body (UB), the medium uterine horn (MH) and proximal uterine horn (PH) and sutured to surrounding tissue. The needle was removed after placing and suturing the electrodes in the correct position. The electrode cables were exteriorised via an incision at the right abdominal wall, 5 cm below lateral transverse processes of lumbar vertebrae.

During 18 days EMG recordings were taken twice a day for 30 minutes periods. Daily blood samples later analysed for progesterone quantification by RIA allowed separation between sessions belonging to follicular (progesterone $<0,5$ ng/ml; treatment F) and luteal phases (progesterone $>0,5$ ng/ml; treatment L). After that, oestrus cycle was synchronized with an intravaginal sponge containing fluorogestone acetate (FGA, 40 mg, Chronogest[®], Intervet) during 9 days. Luteolysis and superovulation (treatment S) were induced 24 hours before sponge withdrawal by the administration of 7.5 mg of prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$, Dinolytic[®], Upjohn) and 1500 IU of equine chorionic gonadotrophin (eCG, Intergonan 500[®], Intervet). Forty five days later the animal was submitted to the same treatment of oestrous synchronization and superovulation, plus the administration of the progesterone antagonist Onapristone (Schering AG; treatment SZK)

beginning at sponge withdrawal (treatment SZK). Onapristone was administered at 12 hours intervals, for 48 hours, i.v., in a dose of 1 mg /kg body weight. Prior to injection, Onapristone was dissolved in NaCl with 1N HCL with a pH of 3.0 reaching a final solution of 5 mg/ ml.

In treatments S and SZK, EMG was monitored for 30 minutes periods right after sponge withdrawal and 24 and 28 hours after. Plasma samples daily taken during both assays allowed further identification of EMG records belonging to follicular phase, equivalent to plasma progesterone levels below 0.5 ng/ml.

Ovulations were confirmed through detection of corpora lutea by endoscopies performed 8 days after sponge withdrawal on all treatments.

Uterine mioelectrical activity (EMG) was recorded using a computerized modular system (Lablink V, Coulbourn Instruments, Allentown, USA). The EMG signals were sampled at 40 Hz, band-pass filtered between 1 and 13 Hz and subsequently full-wave rectified. The average value over 25 seconds was digitized and stored with a time signal.

The number of bursts of spike potentials (events) counted in each 30 minutes record were considered the EMG total activity. The number of events in each uterine portion was expressed as the percentage of the total activity in each treatment (F, L, S and SZK). Differences on percentage of total activity between homologous uterine portions in different treatments and between different uterine portions in the same treatment were checked by chi square tests (StatSoft, Inc., 2000, STATISTICA for Windows). Frequency represents the number of potential bursts in the three uterine portions of each treatment recorded over a 10 minutes period.

Results

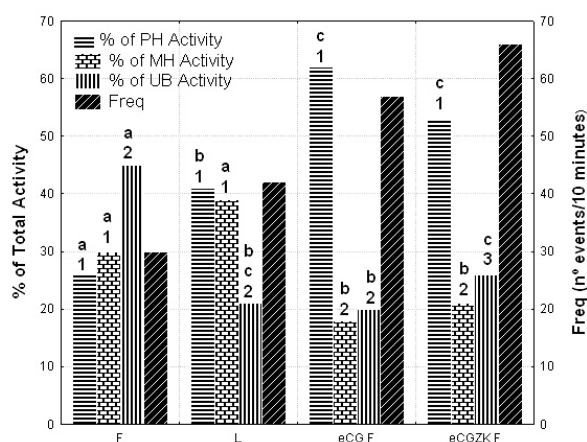
From all records obtained without any treatment, and according to the plasma progesterone levels, one record during follicular phase (F) and one record on luteal phase (L) were considered. From records taken on S and SZK treatments the average of events from records obtained 24 and 28 hours after sponge withdrawal were evaluated, since progesterone levels on plasma samples obtained at those moments were both characteristic of follicular phase (below 0.5 ng/ml).

Analysing the percentage of total activity on each uterine portion from each treatment (graph 1), on F the EMG activity increased backwards (PH<MH<UB) with the UB presenting a significant higher percentage of total activity (44.2%; $P<0.05$). On L the highest percentage of total activity was observed on PH (40.6%) and MH (38.9%), with UB presenting the lowest percentage of total activity (20.6%; $P<0.05$). On S and SZK the highest percentage of total activity was observed on PH (respectively 61.2%, and 52%;

$P < 0.05$) and the lowest on MH (respectively 17.7% and 20.2%). On SZK treatment, UB percentage of total activity (26.8%) was significantly higher than that of MH (20.2%; $p < 0.05$).

Comparing activity of homologous uterine portions between treatments (graph 1), PH percentage of total activity was significantly different between all treatments, with S presenting the higher activity. MH percentage of total activity was significantly higher in F and L treatments. F treatment presented the highest percentage of total activity in UB. Frequency of events was higher on S and SZK than both in F and L.

Graph 1 - Percentage of total activity on each uterine portion and frequency on F, L, S and SZK records



(a,b,c,d) – Differences between homologous uterine portions in different treatments: $P < 0.05$

(1,2,3) – Differences between uterine portions in the same treatment: $P < 0.05$

Discussion and conclusions

The different pattern of activity on F and L phases of oestrous cycle, with F presenting a significant higher activity on the posterior portions of genital tract, and L presenting higher activity on the anterior portions, seems to agree with several authors (Ruckebusch and Bueno, 1976; Garcia-Villar et al., 1982; Prud'Homme and Pele, 1984; Gilbert et al., 1992), according to whom the pattern of uterine motility undergoes marked changes during oestrus cycle. This increase in uterine activity during oestrus supports its presumed function in sperm transport and fertilization (Naaktgeboren et al., 1973; Garcia-Villar et al., 1982; Langendijk et al., 2002a; Langendijk et al., 2002b).

Pattern of uterine myoelectrical activity during follicular phase (F) was modified both by eCG (S) and Onapristone (SZK) treatments. In both treatments it was observed not only an increase on frequency, but also a different pattern of percentage of total activity, with PH presenting the highest percentage of total activity on both treatments. The increase of electrical activity observed in S, when compared with F, agrees

with the results obtained by Prud'Homme and Pele (1984). An increase of uterine myoelectrical activity as well as a predominant backward activity was also observed by others (Arkaravichien and Kendle, 1992; Bouftila and Clabaut, 2001) after administering another progesterone antagonist (RU486), respectively to pregnant mice before implantation and mid pregnant rats. This predominant backward activity, associated with increased activity on PH and the reduction of activity on uterine body 24 hours after sponge withdrawal, which is the time of oestrous and mating (Cavaco Gonçalves et al., 1997), may compromise correct sperm and oocyte transport towards the oviducts, reducing fertilization or embryo collection after superovulation and progesterone antagonists administration as referred by several authors (Whyman and Moore, 1980; Evans and Armstrong, 1984; Hawk et al., 1987; Vinijsanum and Martin, 1990; Yang and Wu, 1990; Juneja and Dobson, 1995; Cavaco Gonçalves and Horta, 1997; Cavaco Gonçalves et al., 1997).

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