In vitro study of bovine uterine contractility at various stages of pregnancy

M. PICCINNO1, A. RIZZO1, M.R. TERLIZZI2, L. MARESCA2, R.L. SCIORSCI1

1 Department of Emergency and Organ Transplantation, Section of Veterinary Medicine and Animal Production, University of Bari Aldo Moro, S.P. per Casamassima km. 3 70010 Valenzano (BA), Italy
2 Free lance Veterinary Doctor

SUMMARY

Introduction - Throughout pregnancy, the uterus does not remain quiescent, but displays contractile activity in several species. This is made possible by the presence of interstitial Cajal cells in the myometrium and by the ability of myometrium to contract/relax even under the action of hormonal or neuronal stimuli.

Aim - This in vitro study investigates spontaneous uterine contractility in cows during different months of pregnancy (from the first at 30 days to the last at 270 days).

Materials and methods - Basal contractility was evaluated in an isolated organ bath and the functionality of strips throughout the experiment was evaluated by a dose of carbachol (10⁻⁵ M), which always had to be repeatable (≤ difference of 20%) with the previous administration of the same substance. Amplitude, frequency and area under the curve (AUC) of contractions for each strip were determined by analysing the sequence corresponding to the last 30 min of contraction, from the recording section.

Results and discussion - This in vitro study demonstrates a variations in contractile capacity, that was highly dependent on the month of bovine pregnancy. Contractility proved extremely limited in early pregnancy (from 0-30 to 90-120 days) and even more so between 120-150 and 180-210 days, reaching maximum force (amplitude) of contraction between 150-180 days. After this period, it decreases from days 180- to 210 and then rises again from days 240- to 270. The contractility detected in the latter two periods is very unusual: between 210-240 days, it is made up of alternating waves with low and high strength, while the period from 240-270 days is characterized by contractures (high-amplitude, low-frequency and long-lasting). The data suggest that the bovine pregnant uterus does not remain quiescent, but displays contractile activity, and raise the hypothesis that steroid hormones may play a role in the modulation of uterine contractions during pregnancy.

Conclusions - Our results provide a new vision of embryonic and fetal mortality, probably attributable to alterations in uterine contractility and cytokine production.

KEY WORDS
Age of pregnancy; uterus contractility; cattle; progesterone; estrogens.

INTRODUCTION

The uterus is the organ of pregnancy, a time when it undergoes profound, largely reversible changes to facilitate foetal growth¹. Hence, during pregnancy, the control exercised by progesterone (P₄), together with metabolic and neural changes, keep it in a relative dormant state². It has been shown that despite P₄ secretion throughout pregnancy, the uterus does not remain quiescent, but displays contractile activity in women, ruminants, monkeys, guinea-pigs, rats and rabbits³. In pregnant cows uterine contractility has been proven in vitro⁴,⁵. Küngid et al.⁶ described the contractions produced by the bovine uterus around parturition through pressure microsensors and electrodes implanted in the pregnant horn. They stated that approximately 3 weeks before calving, the frequency was 0.3 to 0.5 contractions/h, lasting 3 to 30 min, and their amplitude ranged from 60 to 80 mmHg in the caudal part of the uterus, and from 20 to 40 mmHg in its cranial part. In late-pregnant cows, Taverne et al.⁴ confirmed these findings with similar instruments. They used the term “contracture” to describe low-frequency (13.6 ± 0.9 contractures/d), long-lasting (12.1 ± 0.3 min) bursts of uterine activity during the last weeks of pregnancy, and to differentiate them from the contractions distinctive of labour⁴. The onset of these contractions, in several species, is made possible by the presence of interstitial Cajal cells (ICC) in the myometrium⁶. These cells are closely associated with axons, smooth muscle cells, immune cells in capillaries and connective tissue, giving the myometrium the inherent capacity to generate spontaneous contractions of myogenic origin⁷. Indeed, their position would give ICCs an important modulatory role in neurotransmission and immune regulation⁸. Moreover, the myometrium is able to contract/relax even under the action of hormonal or neuronal stimuli that may exert an important contractility-modulating action. In this regard, several in vitro studies highlight the importance of hormones and neurotransmitters on uterine contractions, in pregnant and non-pregnant cows⁹,¹⁰. However, those Authors evaluated the effects of these substances in only a few phases of bovine pregnancy (early- or late-
pregnancy), failing to screen contractility throughout the gestation period.

The aim of this study is thus to evaluate, in vitro, the spontaneous contractility of bovine uterus at different stages of pregnancy (from the first at 30 days to the last at 270 days) in order to understand the physiology of this organ, and provide important background information for the effect of drugs on the bovine pregnant uterus.

MATERIALS AND METHODS

Sixty-three pregnant uteri were obtained from cows slaughtered at a local abattoir. All uteri were found to be free from diseases. Pregnancy has been divided into 9 periods of 30 days each (from 0-30 days to 240-270 days) and 7 strips for each stage of pregnancy (total 63 strips), were considered in our study.

Before stunning, blood samples were collected from the coccygeal vein of each cow in vacutainer glass tubes. Once in the laboratory, blood was centrifuged at 1620g for 10 min at 4°C. The sera were subsequently frozen at -20°C for later analysis of P4, which was conducted with a competitive immunoenzymatic colorimetric method (Progesterone EIA WELL, Radim S.p.A., Italy). The cross-reactions between P4 and steroid hormones were reported as follows: P4 100%; 11-α OH-P4 18%; 17-α OH-P4, 16%; 20-α OH-P4, 1%; estradiol <1x10⁻⁹%; testosterone <1x10⁻⁹%; cortisol <1x10⁻⁹%; cholesterol <1x10⁻⁹%. The detection limit of the assay was 0.16 nmol/l. The intra-assay and inter-assay precision had coefficients of variation of 2.9% and 4.8%, respectively. Estradiol-17β (E₂) concentration was determined by an immunoenzymatic method (Estradiol ELISA, Dia. Metra S.r.l., Italy). The cross-reactions of the antibodies are reported as follows: E₂; 100%; Estrone 2%; Estriol 0.39%; Testosterone 0.02%; Cortisol <7x10⁻⁹%; Progesterone <3x10⁻⁹%; Dhea-s <1x10⁻⁹%. The lowest detectable concentration was 15 pg/ml at the 95% confidence interval.

Gestational age (in days) was recognised by ante and post-mortem examination.

Ante-mortem, pregnancy status was diagnosed, for the first trimester, by clinical examination associated with B-mode ultrasonography (SonoSite MicroMaxx Bothell WA, USA with a 7.5 MHz linear probe), as summarized by Hughes and Davies10.

Post-mortem, the animals’ genital tract was visually examined to confirm the stage of pregnancy (in days) and to identify the gestational age (for those in the last six months). Gestational age was found by measuring the pregnant horn and crown-rump length11.

After slaughter, the uteri were collected in about 20±10 min.

From each uterus, a single circular portion of the middle part of the gravid horn was excised and immediately placed in a flask containing pre-refrigerated Krebs solution (NaCl 113 mM, KCl 4.8 mM, CaCl₂ 2H₂O 2.2 mM, MgSO₄ 1.2 mM, NaH₂PO₄ 1.2 mM, NaHCO₃ 25 mM, glucose 5.5 mM, sodium-ascorbate 5.5 mM), which was prepared daily. Then, it was placed in a water bath at 37°C. After 30 min of constant tension, the last 10 min of contractile activity were recorded using a PowerLab 4/35 (AD Instruments acquisition software). The strips were immediately placed in a jacketed organ bath (mod. 4050 Ugo Basile, Milan, Italy) containing 10 ml of Krebs solution and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The pH was kept at 7.4, and the temperature was maintained at 37°C. A silk thread was used to attach the myometrial strips to a fixed hook and an isometric force displacement transducer (FORT25; AD Instruments, Castle Hill, NSW, Australia). Contractile activities were recorded using a PowerLab 4/35 (AD Instruments acquisition software). During the first 60 min, the strips were allowed to stabilize in the organ baths without applying tension. Subsequently, the strips were allowed to equilibrate under a constant tension of 2 g for about 30 min. After the equilibration period, carbachol (10⁻⁵ M) (Sigma-Aldrich, Milano, Italy), the esterified form of acetylcholine, which has a selective and prolonged contractant effect, was added in a cuvette. This dose, dissolved in Krebs solution, was subsequently removed by washing (wash-out), followed by a period of 30 min or more, needed for the strip to return to baseline. Subsequently, a second dose of carbachol (10⁻⁴ M) was added to the cuvette and its effects were compared to those obtained in the previous administration12.

In the presence of a repeatable response with a deviation ≤±20%, calculated by the formula: (ValueMaximun-Valueminimum)/ValueMaximum*100, we proceeded to test the experimental protocol, otherwise, again after 30 min, a third dose of carbachol was administered at the same concentration (10⁻⁵ M). If this previous administration of carbachol was not repeatable with at least one of the previous doses, the strip was discarded from the experiment12.

Thereafter, regular spontaneous oscillatory contractions were recorded for 30 min. Finally, to evaluate the functionality of the strip throughout the experiment, the registration period, was followed by wash-out and by a dose of carbachol (10⁻⁴ M). The response of the strip had to be repeatable (within 20%) compared to that for the previous administration12.

Amplitude, frequency and area under the curve (AUC) of contractions for each strip were determined by analysing the sequence corresponding to the last 30 min of contraction, from the recording section.

For motility studies, all amplitude, frequency and AUC values were expressed as mean ± SEM and were subjected to statistical analysis by SPSS® Statistics 19 (IBM®, NY). Differences among groups of bovines in the same month of pregnancy were compared using a one-way ANOVA and LSD post hoc test. The values were considered significant for p <0.01. For serum analysis, all values were expressed as Mean ± S.D. Data were analyzed by GLM for repeated measurements, and a post hoc LSD test was applied. A p <0.05 was considered statistically significant.

RESULTS

Spontaneous uterine contractility was observed in 57 strips out of 63 uteri collected. Six strips showing no spontaneous or comparable responses to carbachol (10⁻⁴ M) were discarded.

All strips selected showed regular spontaneous contractions after 30 min of constant tension. The last 10 min of contrac-
tion recording, for all stages of pregnancy, are reported in Fig. 1, such as a representative tracings.

The mean amplitude, frequency and AUC values ± SEM of spontaneous contractile activity (30 min) in pregnant bovine, during the experimental period, are reported in Fig. 2, 3 and 4.

Contractility proved extremely limited in early pregnancy (from 0-30 to 90-120 days) and even more so between 120-150 and 180-210 days, reaching maximum force (amplitude) of contraction between 150-180 days. After this period, it decreases from days 180- to 210 and then rises again from days 240- to 270 (Fig. 1 and 2). The contractility detected in the latter two periods is very unusual: between 210-240 days, it is made up of alternating waves with low and high strength, while the period from 240-270 days is characterized by contractures (high-amplitude, low-frequency and long-lasting) (Fig. 1).

Mean contraction frequency was significantly different in the nine groups and lowest again at 90-120 days, while it increased during the latter periods tested in our study (from 180-210 days to 240-270 days), with the highest values coming at 180-210 days (Figs. 1 and 3).

By contrast, AUC follows the same trend as that described for the amplitude of contraction (Fig. 4).

Serum analyses were in accordance with a previous study. The results showed a higher Estradiol-17β/Progesterone ratio between 90-120 days and 150-180 days (Table 1).

**DISCUSSION**

Many substances (hormones and neurotransmitters) can modulate uterine contractility in non-pregnant and pregnant cows. However, although spontaneous and induced contractility have been well analyzed in non-pregnant cows (follicular and luteal phases, healthy subjects and animals with endometritis), few studies have reported results for pregnant cows. Indeed, to our knowledge, this is the first study that has evaluated the spontaneous contractility of bovine uterus, during every single month of pregnancy (from the first 30 days to 270 days).

Our results showed that, in all of the periods analyzed, there was spontaneous uterine contractility, with contractions varying in strength and duration. Indeed, myometrial activity proved extremely limited in early pregnancy (up to 120 days) and even more in mid-pregnancy (from 120-150 to 180-210 days), achieving of its most impressive contractions at 150-180 days. After this period, uterine contractility de-
myometrial activity and it is generally accepted that estrogen promotes uterine activity whereas P4 favors uterine quiescence through the stimulation or the reduction of gap-junctions. Indeed, the distribution of these intercellular communications is closely related to hormonal changes (estrogen/P4 ratio)14,15. However, we showed that P4, Estradiol-17β and Estradiol-17β/P4 ratio, in peripheral blood, are maintained relatively constant in early-pregnancy and that these values undergo changes from mid-pregnancy. In fact, during the second trimester, Estradiol-17β levels rise slightly from about 90-120 days to 150-180 days, and then drop slightly at around 180-210 days. After this time, Estradiol-17β levels rise again at 240-270 days (Table 1). These results are in agreement with other studies in which steroid hormone concentrations in maternal blood and placental fluids are reported8,17. These systemic and local concentrations of steroid hormones are probably essential for implantation and placentation19 and for adjusting the histotrophic environment necessary for conceptus growth and development20. This hormonal milieu could justify the presence of changes in myometrial contractile activity, during the different stages of bovine pregnancy. In the presence of a stable Estradiol-17β/P4 ratio, the contractility of the pregnant uterine horn is very limited. In this trimester of pregnancy, this low contractility could probably allow for complete placental insertion. Instead, when the Estradiol-17β/P4 ratio starts to increase, there is a slow rise in contractility, that becomes evident only at 120-150 days and reaches its peak at 150-180 days. Indeed, in addition to depolarizing membranes, estrogens favour the up-regulation of oxytocin receptors21. In fact, it has been shown that, around days 60-90 of pregnancy, there is an increase in the number of oxytocin receptors in bovine myometrium, which plateaus at 150-180 days and remains unchanged until a few days before calving22.

This transitory increase in contractility is physiological and promotes placental exchanges and placental function, that are very important to foetal development. However, it could be extremely dangerous, because any stress or infection in...
lease of the foetal membrane

However, it is possible to believe that the increase in contractility in the later stages may be due to the gradual increase in alpha2-adrenergic receptors. Indeed, the bovine uterus is extremely rich in alpha2-adrenergic receptors with excitatory activity, whose concentration rises in the presence of high estrogen level26,27. This is extremely important from a clinical point of view, and particularly in the choice of sedative in pregnant cattle surgery. For example, the use of xylazine, a known alpha2-agonist, could increase uterine contractility and, subsequently, increase the risk of abortion.

CONCLUSIONS

In conclusion, our study is the first work to show contractility in a pregnant uterus in the different months of gestation. It showed variable contractility during pregnancy, with the lowest values at 90-120 days and the highest at 150-180 days. These results explain the importance of several factors, in particularly steroid hormones, in pregnancy (P4 in early-pregnancy and estrogens in late-pregnancy) and provide a new key to understanding the causes of abortion.

This study serves as an aid to the clinician in the choice of sedative to use for surgery in pregnant subjects. Further studies are, however, necessary to clarify the relationship between the contractility detected in this study and the concentration of alpha2-adrenoreceptors.

References

In vitro study of bovine uterine contractility at various stages of pregnancy


