

# ***Ex vivo* influence of carbetocin on equine myometrial muscles and comparison with oxytocin**

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## **Abstract**

In order to determine the inter-cyclic effect of oxytocin and carbetocin on equine myometrial tissue, the effect of the drugs was evaluated through pharmacokinetic and pharmaco-dynamic studies. The complete pharmacokinetic profile for oxytocin was unknown and had to be established. To do so, 25 IU of oxytocin were administered intravenously to six cycling mares and blood samples were collected before and 2, 4, 8,

and 15 min after administration. The half-life of oxytocin was determined to be 5.89 min, the clearance rate 11.67 l/min, MRT 7.78 min,  $T_{max}$  2 min, and  $C_{max}$  0.51 ng/ml. The effective plasma concentration was estimated to be 0.25 ng/ml. This was similar to the concentration achieved for the organ bath study where the  $EC_{50}$  was calculated at 0.45 ng/ml. To determine the inter-cyclic effect of oxytocin and carbetocin uterine myometrial samples were collected from slaughtered mares in oestrus, dioestrus and anoestrus. The samples were mounted in organ baths and exposed to four ascending, cumulative doses of oxytocin and carbetocin. Area under the curve and amplitude,  $E_{max}$  and  $EC_{50}$  were studied for each agonist and statistically evaluated. The effect of oxytocin on equine myometrial tissue was higher during dioestrus, and surprisingly anoestrus, than during oestrus, whereas the effect of carbetocin was the same independent of the stage of oestrous cycle. A significant difference was found for oestrous and anoestrous samples when oxytocin was used but not when carbetocin was used.

*Keywords:* equine myometrium, oxytocin, carbetocin, *ex vivo*, organ bath

## 1. Introduction

Two major causes for economic losses in the equine breeding industry are the failure of a mare to conceive or a delay in a mare's re-conception [1, 2], both of which have shown to be caused mainly by a delayed uterine clearance in association with reduced uterine contractility [2]. Uterine contractions are vital in the transport of sperm through the uterus to the uterotubal junction, as well as for the clearance of sperm after breeding, or of accumulated intrauterine fluids [3]. A large percentage of mares susceptible to endometritis most likely have intrinsic contractile defects in myometrial contractility [4]. This results in accumulation of intrauterine fluid after breeding, predisposing the mare to persistent mating induced endometritis (PMIE)[4]. In the mare, treatment of PMIE and stimulation of postpartum uterine involution [5] includes the use of oxytocin and natural or synthetic prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ), which aid in promoting uterine clearance. Oxytocin stimulates uterine contractions, release of arachidonic acid and prostaglandin formation, which in turn enhance uterine contractions by causing membrane depolarization and by increasing the number of gap junctions [6]. Clearance of uterine contents provides a uterine environment suitable for an embryo descending into the uterus, thus improving conception rates.

Both oxytocin and  $PGF_{2\alpha}$  have disadvantages in their application; for example, oxytocin needs to be given in short intervals because of its short half-life (6.8 min.), and  $PGF_{2\alpha}$  in its natural form has unwanted side effects (sweating, tachypnea, increased gastrointestinal motility, signs of colic) [7, 8]. A drug without side effects and a long half-life stimulating a prolonged tocolytic effect might improve uterine clearance. The

use of carbetocin, a long acting analogue of oxytocin, has been investigated as a beneficial form of treatment on uterine involution in bovines [9], as well as in humans for the treatment of postpartum haemorrhage [10, 11, 12]. In adult, non-lactating anoestrous mares carbetocin has been shown to have a half-life of approximately 17 min., which is 2.5-fold longer than that of oxytocin, and not associated with any side effects [7]. As such we believe that carbetocin may prove more beneficial in horses in inducing parturition, decreasing the time to conception post-partum, and aid in the treatment of PMIE [13].

For the current study we investigated if the concentration-dependent contractile responses of carbetocin in healthy oestrous, dioestrous and anoestrous equine myometrial smooth muscle were superior to that of oxytocin. The duration of effect was extrapolated from the pharmacokinetic time versus concentration profile for the mentioned oxytocin (self-generated) and carbetocin [7].

## 2. Materials and Methods

### 2.1. Pharmacokinetic Study

The pharmacokinetics of oxytocin was evaluated following a single intravenous dose in adult horses.

#### 2.1.1. Animals

Six healthy, adult, non-lactating cycling Nooitgedacht mares of the Veterinary Faculty

were used for the study. All animals were vaccinated and dewormed, and kept in their usual environment. The study was approved by the Animal Use and Care Committee of the University of Pretoria according the South African standard for the care of laboratory animals (SANS 10386, Project number V026/08).

#### 2.1.2. Sample collection and Preparation

An indwelling intravenous catheter was placed into the left jugular vein for blood sampling. The oxytocin treatment (25 IU) (Fentocin<sup>®</sup>, Virbac, South Africa) was administered into the opposite jugular vein by means of a disposable syringe and a 20 G needle. Blood samples were collected before administration and at 2, 4, 8 and 15 minutes after oxytocin administration, into chilled serum tubes containing aprotonin (500 KIU/ml of blood) (Aprotonin, Sigma-Aldrich (Pty) Ltd., PO Box 10434, Aston Manor 1630, South Africa).

Following collection, the blood samples were centrifuged at 1600 g at 4 °C for 15 min and the supernatant of each sample was transferred to a labelled polycarbonate tube and immediately frozen at -80 °C. Hormone assay samples were extracted using a 200 mg C18 Sep-Pak column under the following conditions: equilibration of the column with 1 ml of acetonitrile, followed by 10-25 ml of 0.1% trifluoroacetic acid (TFA) in water prior to application of the sample. After sample application, the sample was washed with 10-20 ml of 0.1% TFA in water, followed by a final elution with 3 ml of acetonitrile 0.1% TFA in water (60:40). Samples were subsequently dried under a steady stream of nitrogen at 40°C.

Oxytocin concentrations were quantified using the Oxytocin Enzyme Immunoassay Kit (Assay Designs, 5777 Hines Drive, Ann Arbor, MI 48108, USA) according to the manufacturer's instructions. In short, 100  $\mu$ l of sample were pipetted into sample wells with 50  $\mu$ l of both the blue conjugate (alkaline phosphatase conjugated with oxytocin) and antibody. The plates were subsequently gently mixed and incubated at 4 °C for 18-24 hours. Following three washes with 400  $\mu$ l of wash solution 5  $\mu$ l of blue conjugate was added into to wells with 200  $\mu$ l of pNpp Substrate (p-nitrophenylphosphate in buffer) and allowed to incubate at room temperature for 1 hour prior to the addition of 50  $\mu$ l of Stop Solution. The 96 well plate was immediately read with an Eliza iEMS reader MF (Labsystems, P.O. Box 208, FIN-00811 Helsinki, Finland; wavelength range 340-850 nm, wavelength accuracy  $\pm$ 2 nm) at an optical density of 405 nm with correction between 570 and 590 nm.

### 2.1.3. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using Kinetica Version 5.0 (Thermo). The area under the concentration–time curve to the last plasma sampling time ( $AUC_{last}$ ) was calculated according to the linear trapezoidal rule. The area under the concentration–time curve extrapolated to infinity ( $AUC_{inf}$ ) as  $AUC_{inf} + C_t/L_z$ , where  $C_t$  was the last measured concentration and  $L_z$  was the slope of the terminal proportion of the plasma versus time concentration curve following natural logarithmic transmotion. The half-life ( $t_{1/2}$ ), mean residence time (MRT), volume of distribution (l/kg) and clearance (ml/kg/min) were calculated using standard formulae.

## 2.2. Organ bath myometrial study

*Ex vivo* influence of carbetocin on equine myometrial muscles and comparison to oxytocin

### 2.2.1. Sample preparation

Myometrial smooth muscle strips were obtained from 16 unmated cross-breed Nootgedacht mares in oestrus (n=5), dioestrus (n=6), and anoestrus (n=5) between the age of three and ten years from a commercial abattoir (Krugersdorp, South Africa). Stage of cycle was determined on examination of the ovaries and plasma progesterone concentrations (PPC). The presence of a corpus luteum and high progesterone concentration confirmed dioestrus. A regressing corpus luteum, a low progesterone concentration, and a dominant follicle of more than 2 cm confirmed oestrus. Inactive ovaries without a dominant follicle and a corpus luteum and low progesterone concentration confirmed anoestrus.

### 2.2.2. Sample collection

A blood sample for PPC was collected in 5 ml heparin tubes during the slaughter of the animal, centrifuged and the supernatant frozen for later analysis. The entire reproductive tract, including vagina, cervix, uterine body, uterine horns and both ovaries, were obtained immediately after slaughter. A four cm rectangular specimen was sectioned from the base of each uterine horn and placed into 4 °C cold Krebs-Henseleit solution (KHS) which was preoxygenated for 30 min with 95% O<sub>2</sub> and 5% CO<sub>2</sub> in oxygen (carbogen) to reach a pH of 7.3-7.4. The KHS contained: NaCl 6.9 g/l; KCl 0.35 g/l;

NaHCO<sub>3</sub> 2.09 g/l; MgSO<sub>4</sub> 0.14 g/l; KH<sub>2</sub>PO<sub>4</sub> 0.16 g/l; Glucose 1.09 g/l; CaCl<sub>2</sub>·2H<sub>2</sub>O 0.28 g/l and Na-Pyruvate 0.22 g/l and was made fresh prior to sample collection (all chemicals procured from Sigma-Aldrich, South Africa). The samples were subsequently transported to the Faculty of Veterinary Science.

A uterine swab was taken from each mare and the smear examined for uterine pathology, such as the presence of neutrophils and bacteria indicating uterine inflammation. A full thickness uterine biopsy was taken from each mare for histopathology and evaluated according to Kenney [14]. This system takes into consideration inflammation and fibrosis of the endometrium and provides an estimation of the mare's ability to conceive and maintain a pregnancy until term. The uterus was graded from Grade I, IIA, IIB to III. Mares with a Grade I have a more than 80% chance of conceiving and maintaining a pregnancy until term whereas mares with a Grade III are expected to only have a 10-50% of conceiving and maintaining a pregnancy until term. Only mares without uterine pathology and an endometrial biopsy score of I and IIA were included in the study.

### 2.2.3. Preparation of smooth muscle specimen

Following transportation to the laboratory, the 32 tissue samples were sectioned into 5 x 20 mm strips in warmed oxygenated KHS. All the prepared strips were full thickness and cut parallel to the longitudinal muscle fibres. The endometrium and any excess connective tissue were dissected apart from the muscle layers using a dissecting microscope.

#### 2.2.4. Tissue baths

The 32 tissue samples were mounted in standard 100 ml organ baths. Each bath was filled with 50 ml of KHS, maintained at 37 °C and continuously bubbled with carbogen.

A force-displacement transducer attached to a chart recorder was used to evaluate the mechanical activity of the myometrium. Time from collection to tissue mounting was approximately 90 to 100 min. Tissue strips were allowed to equilibrate for 30 min prior to tension being applied. Tissues were subsequently mounted under tension of two grams [15], where after they were exposed to another 15 min of equilibration prior to initial stimulation.

Each muscle strip was exposed to four ascending concentration doses of oxytocin (0.086 (Conc. 1), 0.94 (Conc. 2), 9.5 (Conc. 3) and 52.3 ng/ml (Conc. 4)) and carbetocin (3.5 (Conc. 1), 38.5 (Conc. 2), 388.5 (Conc. 3), and 2138 ng/ml (Conc. 4)) (V-Tech Pharmacy, Midrand, South Africa) in a cumulative concentration-response protocol.

Tissues were allowed 2 minutes to contract before ascension to the next dose. After the response to the additive last concentration was recorded, the compound was washed from the organ bath three times, before the subsequent assays were run. The tissues returned to their normal resting phase after the washes and the experiment was continued. Needed electrolytes were supplied in the Buffer solution to avoid depletion of electrolytes. The tissues were given time to equilibrate between responses to return to no or initial contractility.

#### 2.2.5. Data analysis

The amplitudes of the myometrial contractile activity were converted to % response

according the following equation: % response = amplitude achieved after exposure/highest amplitude achieved (all samples) multiplied by 100. The maximum response ( $E_{max}$ ) and the concentration that produced 50% of the maximum effect ( $EC_{50}$ ) were ascertained by curve fitting the dose response data to an  $E_{max}$  equation (Kinetic 5, Thermo).  $EC_{50}$  is the concentration required to produce 50% of the response and can be used for comparison between drugs to the nature of the linear nature of PD graphs from 20 to 80%. The  $E_{max}$  is theoretically the concentration required to produce a maximum effect and is based on curve fitting. Due to the sigmoid nature of OD curves the  $E_{50}$  is never related in a linear manner to  $E_{max}$ . The difference in response for the various cycles was evaluated using an ANOVA with Tukey HSD and Bonferroni post-hoc test. Statistical analysis was performed with the statistical software SPSS19 (IBM). A Kolmogorov-Smirnov and Lilliefors table was used to assess the normality of distribution following various transformations. Since normality could not be demonstrated, differences per treatment for the different cycles were compared with a Kruskal Wallis test and a p-value of 0.05 Post-hoc comparison were made between the cycles using a Mann-Whitney test with the p-value being set at 0.025 following Bonferroni correction. Differences between the different treatments per cycle were compared using a Mann-Whitney test at a p-value of 0.05.

### 3. Results

#### 3.1. Pharmacokinetic study

The plasma versus time concentration profile obtained for oxytocin during the study is

presented in Figure 1. The calculated pharmacokinetic parameters are presented in Table 1.

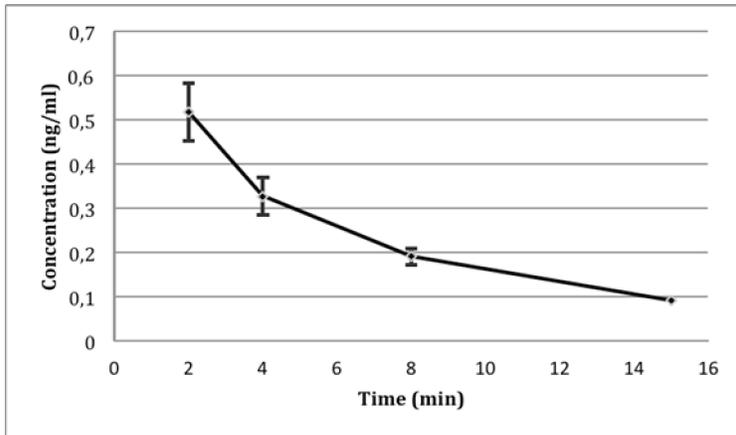


Figure 1 Average plasma concentration versus time profile for horses after intravenous administration of 25 IU oxytocin (Fentocin®)

All the horses had baseline oxytocin concentrations of  $0.009 \pm 0.004$  ng/ml. Following administration oxytocin was characterized by a mean plasma concentration of  $0.51 \pm 0.01$  ng/ml. The plasma concentration thereafter rapidly declined with the half-life of elimination of  $5.89 \pm 0.699$  minutes. The drug was also characterized by a low MRT of  $7.708 \pm 1.15$  minutes.

**Table 1****Plasma pharmacokinetic parameter for oxytocin following a single intravenous injection of 25 IU to six Nooitgedacht mares**

Animal	AUC <sub>last</sub>	AUC <sub>inf</sub>	Lz	AUMC <sub>last</sub>	t <sub>1/2</sub>	MRT	Clearance	V <sub>ss</sub>	V <sub>z</sub>
	ng/mL*min	ng/mL*min	L/min	ng/mL/min <sup>2</sup>	min	min	L/min*kg	L/kg	L/kg
1	5,080	6,020	0.12	24,300	5.84	7.68	9.14	70.25	77.07
2	3,550	4,430	0.10	18,400	6.44	8.97	12.40	111.24	115.27
3	4,540	5,340	0.12	21,500	5.77	7.55	10.29	77.70	85.70
4	3,690	4,640	0.10	19,700	6.63	9.26	11.83	109.61	113.25
5	3,710	4,320	0.11	15,400	6.02	6.94	12.70	88.25	110.44
6	3,600	4,010	0.14	16,100	4.64	6.26	13.70	85.78	91.81
Mean	4,030	4,790	0.11	19,200	5.89	7.78	11.67	90.47	98.92
Median	3,700	4,540	0.11	19,000	5.93	7.62	12.11	87.02	101.13
SD	630	745	0.01	3,350	0.69	1.15	1.67	16.71	16.17
% CV	15.63	15.55	9.09	17.44	11.71	14.78	14.31	18.47	16.89

AUC<sub>last</sub>: area under the concentration–time curve to the last plasma sampling time, AUC<sub>inf</sub>: area under the concentration–time curve extrapolated to infinity, Lz: slope of the curve=slope of the linear regression of the log-transformed concentration–time, AUMC<sub>last</sub>: Area under the moment curve to the last sampling time, t<sub>1/2</sub>: half life of elimination, MRT: mean residence time, V<sub>ss</sub>: Volume of distribution at steady state; V<sub>z</sub>: Volume of distribution at the terminal stage

### 3.2. Organ bath myometrial study

From the 16 mares used for the study, five mares were in oestrus, six in dioestrus and five in anoestrus. Uterine swabs showed no signs of infections, while all uterine biopsies were scored as Grade I or Grade IIA. From each of the 16 mares, two strips of uterine tissue, including longitudinal and circular muscle layers, were used for the study. Of the 32 samples only 14 (44%) showed spontaneous uterine contractions. Five of the samples showing spontaneous contractions were obtained from mares in oestrus, two samples from mares in dioestrus, and seven samples from mares in anoestrus.

#### 3.2.1. EC<sub>50</sub> values for oxytocin and carbetocin

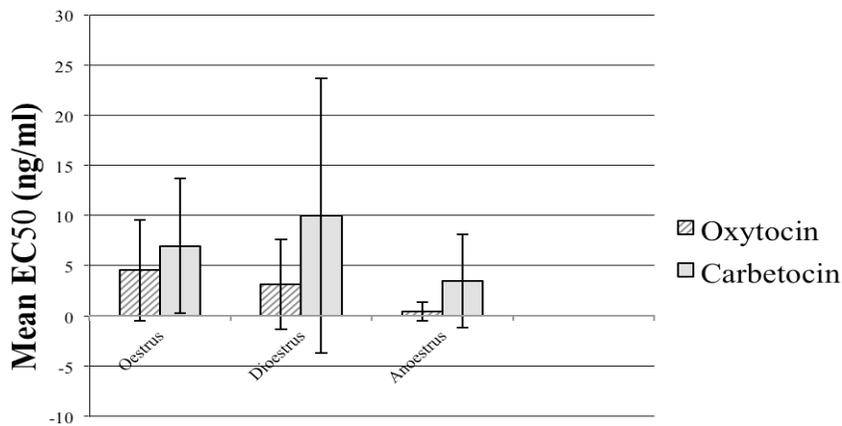


Figure 2 Effect of oxytocin and carbetocin on oestrous (n=10), dioestrous (n=12), and anoestrous (n=10) myometrial tissue described as mean EC<sub>50</sub> (ng/ml;  $\pm$  SD). Significant difference between stages only for oxytocin: oestrus and anoestrus (P=0.008), dioestrus and anoestrus (P=0.009)

Figure 2 shows the EC<sub>50</sub> values (ng/ml) for oxytocin and carbetocin for mares in oestrus,

dioestrus and anoestrus. Mean  $\pm$  S.D. for oxytocin EC<sub>50</sub> values were 4.57  $\pm$  5, 3.12  $\pm$  4.47, 0.45  $\pm$  0.92 ng/ml for oestrous, dioestrus and anoestrous mares. Mean  $\pm$  S.D. for carbetocin EC<sub>50</sub> values were 6.95  $\pm$  6.73, 10.51  $\pm$  13.68, 3.47  $\pm$  4.65 ng/ml for oestrous, dioestrus and anoestrous mares.

No significant difference could be observed between any of the oestrous cycle phases for carbetocin (p=0.79). For oxytocin a significant difference was present between stages (P=0.007): oestrus and anoestrus (P=0.008), and dioestrus and anoestrus (P=0.009), while no significant difference was present between oestrus and dioestrus (P=0.425).

Comparison between oxytocin and carbetocin during the different cycle stages revealed a significant difference for anoestrus (p=0.031) but no significant difference for oestrus and dioestrus.

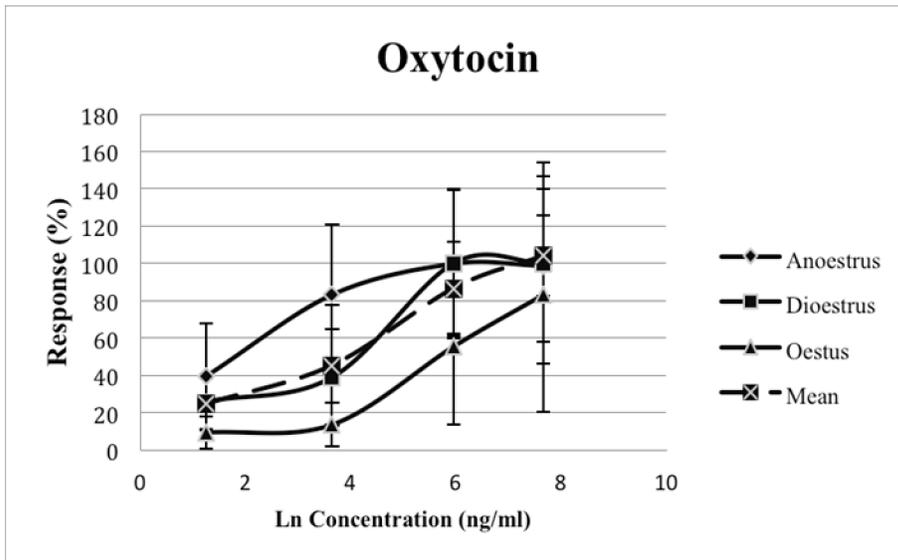


Figure 3 Effect of cycle stage on myometrial response to oxytocin stimulation *ex vivo* (oestrus n=10, dioestrus n=12, anoestrus n=10,  $\pm$ SD).

Figure 3 shows the effect of oxytocin on equine myometrial tissue *ex vivo* at different stages of the oestrous cycle. A weak relative response of oestrous myometrial tissue can

be observed for Conc. 1 and 2. There after the relative response increases for Conc. 3 and Conc. 4 in a dose-dependent fashion. During dioestrus, a similar myometrial response can be observed but with an overall stronger relative response of the myometrial tissue to oxytocin, compared to oestrous myometrial tissue. During anoestrus, a dose dependent increase in response can be observed with increasing concentrations, up to Conc. 3 where-after the relative response of the myometrial tissue stays the same or starts to decline with Conc. 4 indicating that the maximum achievable effect has been reached. The mean relative response of oxytocin shows a steady increase with increasing drug concentrations.

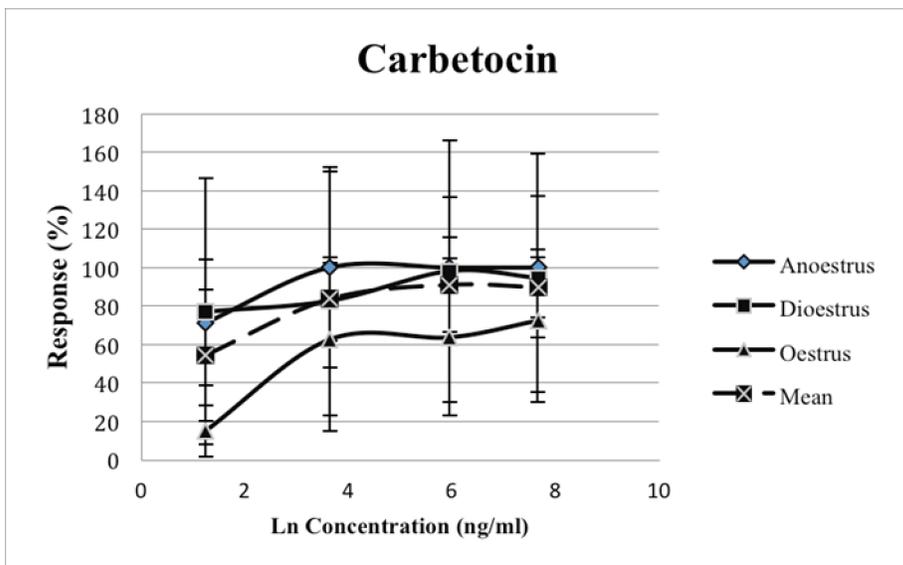


Figure 4 Effect of cycle stage on myometrial response to carbetocin stimulation *ex vivo* (oestrus n=10, dioestrus n=12, anoestrus n=10,  $\pm$ SD)

Figure 4 shows the effect of carbetocin on equine myometrial tissue *ex vivo* at different stages of the oestrous cycle. The relative response of oestrous myometrial tissue to carbetocin is weak for Conc. 1, stronger for Conc. 2, 3, and 4 but with no dose dependent increase for these concentrations. A stronger relative response to carbetocin can be

observed for dioestrous and anoestrous myometrial tissue compared to oestrous tissue, especially in response to Conc. 1.

The mean relative response of carbetocin shows a dose dependent increase up to Conc. 2, were after the effect plateaus.

#### 4. Discussion

In order to determine the inter-cyclic effect of carbetocin and oxytocin, the effect of the drugs was evaluated through pharmacokinetic and pharmaco-dynamic studies. The effects of both drugs in organ baths were evaluated. To determine the validity of the results, the  $EC_{50}$ 's obtained were compared to estimated effective plasma concentration. Since the complete pharmacokinetic profile for oxytocin was unknown the first part of modelling was to establish the pharmacokinetics of oxytocin. For the current study the half-life of oxytocin in cycling, non-pregnant mares was determined to be 5.89 minutes, which differed minimally from the half life of 6.8 min reported by Paccamonti *et al.* [23]. The difference in the half-life of the two studies most likely results from the different animals used in the two studies (Warmblood mares *vs.* Nooitgedacht mares) as the difference in body weight and metabolic capacity might affect the clearance rate and the half-life of the drug. This result is supported by other pharmacokinetic studies on oxytocin in farm animals, where a half-life of 22.3 min was reported in goats while the half-life in cattle, from various studies, was approximately 7 to 9 min [24, 25, 26, 27]. As can be seen from this study, there is an inverse proportional relationship between bodyweight and the half-life of response i.e. the smaller animal being the goat having the longer half-life. Another interesting feature of oxytocin is that its metabolism appears to be largely extra-hepatic. Mitchell and co-workers found that rat uterine tissue contains

peptidase activity which degrades, metabolizes and thus inactivates oxytocin. They speculated that this mechanism could be important in regulating the metabolism of oxytocin during parturition [28].

As expected oxytocin was characterised by a low mean residence time (MRT) and a high mean volume of distribution (Vd). Since MRT represented approximately 63% of total time any drug spends in the body, the small value obtained for this study was clearly related to the rapid half-life of the drug. As an estimate the MRT was inversely proportional to the elimination constant. The same principle may be applied to the Vd. The Vd was determined from the dose being administered against the maximum plasma concentration after intravenous administration. Since the maximum concentration achievable was clearly related to half-life, the rapid elimination did result in a falsely elevated Vd. However, another factor that most likely contributed to the high Vd was the ability of oxytocin to be highly tissue-bound and metabolised in the biophase where it was active [28] at the level of oxytocin receptor.

In order to determine the validity of the results achieved from the *ex vivo* study, the results were evaluated using pharmacokinetic-pharmacodynamic modelling.

The use of *ex vivo* techniques to study uterine contractility permits the evaluation of specific contractile responses in tissues obtained from animals of precisely known *in vivo* functional status in the absence of potentially confounding interactions that might occur *in vivo* [16]. *Ex vivo* studies on smooth muscle strips in organ baths have served as an alternative for investigations of myometrial activity. The *ex vivo* assessment of the contractility of the smooth muscle layer of the uterus in the mare may serve as a more precise and accurate measurement for understanding the influence and interaction of endogenous and exogenous drugs under *in vivo* conditions [17, 15]. For this study, the

effect of the selected agonists on the myometrium alone was determined, as this tissue contains the receptor series involved with contractility.

During the initial part of the experiment, spontaneous contraction of equine myometrial tissue could be observed in only 14 out of 32 samples (44%). This was markedly different to a study by Hirsbrunner and co-workers [15], who were able to demonstrate spontaneous myometrial contractions of all oestrous and dioestrous circular and longitudinal myometrial tissue samples, whereas Rigby and co-workers [17] were “seldom” able to, while Ousey *et al.* [18] were not able to demonstrate any spontaneous myometrial contractions. This tends to suggest that the response of the myometrium is dependent on a number of factors such as survivability of tissue *ex vivo*, number of active receptors, muscle activity and individual variation.

The  $EC_{50}$  (amount of drug necessary to achieve 50% of maximal contraction) was found to be highest in oestrous tissues, lower in dioestrous tissues, and lowest in anoestrous myometrial tissues after oxytocin administration. Although the  $EC_{50}$ , due to the sigmoid nature of OD curves, is not related in a linear manner to  $E_{max}$  the effect on oestrous tissue is unexpected as the results do not follow clinical responses for the drug. The high  $EC_{50}$  of oestrous tissue suggests that an increased amount of oxytocin is needed in order to cause a response of the myometrium. Expected was that, due to a high concentration of oxytocin receptors within the myometrium during oestrus, a decreased amount of oxytocin is needed to result in a myometrial response, as suggested by literature [21].

One explanation may involve the rapid metabolism at the level of the endometrium. This mechanism was not considered important for this study, as the endometrium was removed prior to tissue incubation. A second explanation may involve the concentration of active receptors at the level of the muscles. In studies by Tetzke *et al.* [19], it was

clearly demonstrated that the myometrium receptivity for oxytocin depends on the cycle stage. Further studies by Stull and Evans [20] and Sharp *et al.* [21] have demonstrated that the oxytocin receptors are present to a greater extent in the myometrium than in the endometrium of non-pregnant and pregnant mares. Receptor numbers have also been shown to differ within the oestrous cycle itself as the highest concentration of receptors can be found during early oestrus (day 14 to 17), compared to late oestrus (day 18 to 0), and dioestrus (day 1 to 13) [21]. The oestrous tissue used in this study had been collected from mares in advanced oestrus (day 18 to 0) during which the receptor number has found to be lower than in early oestrus, increasing the  $EC_{50}$  to some degree. It arises the question if a higher receptor concentration will result in a higher response, and how will this be evident in the animal *in vivo*? During an *ex vivo* study no inhibitory systems are in place as would be *in vivo*, which might influence the results. No literature could be found with regards to the response of myometrial tissue to oxytocin during anoestrus. It is surprising that anoestrous myometrial tissue should have a significantly lower  $EC_{50}$  than oestrous and dioestrous tissue and thus a higher response to oxytocin, assumingly due to a higher oxytocin receptor concentration. These results can currently not be explained and need further investigation.

In contrast, myometrial contractility in response to carbetocin did not appear to be affected by the stage of the cycle. The reason for the lack of a stage related response by carbetocin is more difficult to explain. One reason for the effect seen may be due to a difference in receptor binding between oxytocin and carbetocin. The first receptor mechanism results from the direct stimulation of the myometrium via a vasopressin receptor with subsequent activation of a calcium controlling second messenger system [22]. The second receptor mechanism relies on the stimulation of oxytocin receptors in

the endometrium and myometrium with subsequent release of  $\text{PGF}_{2\alpha}$  and its related stimulation of uterine contractions and luteolysis [22]. Of these two receptor series the oxytocin receptor is cyclic (as mention above) in their concentration while the vasopressin receptor remains at a constant level [22]. In a study by Engstrom *et al.* [22] it was further demonstrated that oxytocin had poor affinity for the vasopressin receptor while carbetocin had affinity for both receptor series. This would therefore explain the differences evident i.e. the effect of oxytocin being cyclic due to cyclic activation of the receptor while carbetocin is able to produce a constant effect due to the constant concentration and presence of the vasopressin receptors system [22].

Based on the pharmacokinetic profile for oxytocin and the known effective duration of 6.8 min for the non-pregnant mare, the effective plasma concentration is estimated to be 0.25 ng/ml. This was similar to the concentration achieved for the organ bath study where the lowest  $\text{EC}_{50}$  was calculated at 0.45 ng/ml for anoestrous mares. When the same trend is applied to the published carbetocin pharmacokinetic information, and the effective duration of 17 min, the effective plasma concentration was estimated to be 3 ng/ml. This was once again familiar to the lowest  $\text{EC}_{50}$  of 3.47 ng/ml in anoestrous mares. This therefore tends to suggest that the organ bath concentrations are relevant to the adult horse.

## 5. Conclusion

The results suggest that the effect of oxytocin on equine myometrial tissue is higher during dioestrus, and surprisingly anoestrus, than during oestrus, whereas the effect of carbetocin seems to be the same independent of the stage of oestrous cycle. An

advantage of carbetocin is its longer half-life (17 min) over the half-life of oxytocin (6 min) in the mare.

These results might be useful in the treatment of uterine pathology, such as advanced stages of PMIE, pyometra as well as for induction or enhancement of myometrial contractions during parturition.

#### Conflict of interest

None of the authors have any conflict of interest to declare.

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