

Endocrine responses to standardized cervical manipulation in the mare

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Summary

The impact of manipulations of the equine cervix on the endocrine system, oestrous cycle patterns, pregnancy rates and embryonic development was investigated by six studies in cyclic and inseminated pregnant mares. In cyclic mares, standardized cervical dilation at Day 7 after ovulation (Day 0 = ovulation) shortened luteal phase and oestrous cycle by almost 2 days. Cervical dilation did not provoke an immediate release of PGF2 α , but stimulated a distinct secretion of oxytocin. Starting from Day 10, dilated mares showed significantly lower progesterone levels and went two days earlier into luteolysis compared with controls. Administration of xylazine hydrochloride blocked the oxytocin release completely and oestrous cycle length was not altered by combined treatments. In inseminated, pregnant mares, dilation of the equine cervix did not affect pregnancy rates and early embryonic growth rates. Cervical manipulation also stimulated the secretion of oxytocin, but plasma levels of progesterone did not vary among groups. In contrast, dilated mares treated with xylazine hydrochloride did not secrete oxytocin, but progesterone levels tended to be lower compared with untreated controls (xylazine hydrochloride only). Surprisingly, embryonic growth rates were significantly higher in dilated mares pretreated with xylazine hydrochloride compared with controls. Cervical dilation did not change plasma concentration patterns of LH and prolactin with one exception. Only one pregnant mare showed a distinct rise of prolactin after cervical dilation. Moreover, this mare was the only one, which developed endometrial inflammation and subsequent embryonic death by Day 12. This observation needs to be investigated further for clarification of a possible contribution of prolactin to the mechanisms involved. Additionally, the role of xylazine has to be evaluated, because there is no further evidence from our data that the inhibition of oxytocin release is of significance concerning the effects on embryonic growth by combined treatment of cervical dilation and xylazine hydrochloride. In conclusion, the results of our studies showed that cervical manipulation has almost no or little negative impact on pregnancy rate and early embryonic growth in mares.

Keywords: mare, cervical dilation, oestrous cycle, early pregnancy, reproduction

Endokrine Reaktionen auf eine standardisierte Zervixmanipulation bei der Stute

Der Einfluss der Zervixmanipulation auf das endokrine System, Verlauf des Sexualzyklus, Trächtigkeitsraten und Embryonalentwicklung wurde in sechs Studien bei zyklischen und graviden Stuten untersucht. Bei zyklischen Stuten verkürzte die standardisierte Dilatation des Zervikalkanals am Tag 7 (Tag 0 = Tag der Ovulation) die Dauer der Lutealphase und des Sexualzyklus um etwa zwei Tage. Die Dilatation der Zervix löste aber keine Sekretion von PGF2 α , dafür aber eine deutliche Freisetzung von Oxytozin aus. Ab Tag 10 zeigten die dilatierten Stuten signifikant geringere Progesteronwerte als die Kontrollgruppe, und bei ihnen begann dann auch die Luteolyse um etwa zwei Tage früher. Die Verabreichung von Xylazinhydrochlorid blockierte die Oxytozinfreisetzung zur Gänze, und die Wirkung auf die Zyklusdauer blieb aus. Bei den gesamten, trächtigen Stuten beeinflusste die Zervixdilatation die Trächtigkeitsrate und das embryonale Wachstum nicht. Die Dilatation stimulierte auch die Sekretion von Oxytozin, hatte aber keinen Einfluss auf die Progesteronkonzentration. Im Gegensatz dazu kam es nach zusätzlicher Verabreichung von Xylazin zu keiner Freisetzung von Oxytozin, aber die Progesteronwerte der dilatierten Stuten schienen geringer zu sein im Vergleich zu der Kontrollgruppe. Überraschenderweise war das embryonale Wachstum nach Zervixdilatation und Xylazin signifikant höher als in der Kontrollgruppe. Die Manipulation der Zervix führte zu keiner Veränderung der Sekretion von LH und Prolaktin, mit Ausnahme einer Stute, die einen deutlichen Prolaktinanstieg nach Dilatation der Zervix aufwies. Dieses Tier entwickelte in weiterer Folge eine Entzündung des Endometriums und Absterben des Konzepts am Tag 12. Diese Beobachtung macht noch weitere Untersuchungen zur genauen Abklärung der biologischen Bedeutung notwendig. Darüber hinaus muss auch die Rolle von Xylazin evaluiert werden, weil aus unseren Daten hervorgeht, dass die Hemmung der Oxytozinsekretion nicht direkt am Zustandekommen der Effekte der kombinierten Anwendung von Zervixdilatation und Xylazin beteiligt ist. Zusammenfassend kann festgestellt werden, dass die Zervixmanipulation einen geringen oder keinen Einfluss auf die Trächtigkeit und das frühembryonale Wachstum hat.

Schlüsselwörter: Stute, Zervixdilatation, Sexualzyklus, Frühträchtigkeit, Reproduktion

Introduction

Manipulations of the cervix are a prerequisite for numerous diagnostic and therapeutic techniques in equine gynaecology, obstetrics and reproductive biotechnology such as endometrial swabs, cytology and biopsies, palpation of the vaginal portion of the cervix, intrauterine insemination and instillation, therapeutic uterine flushing, embryo collection and transfer, and surgery of the cervix. Depending on the reproductive status of the mare, knowledge of the effects of cervical manipulations would allow avoiding negative consequences for the reproductive success in mares.

The effects of cervical manipulations on oestrous cycle and early pregnancy in the mare have been under discussion for decades. Insertion of aluminum rods into the cervical canal did not change oestrous cycle patterns (Arthur 1975). In contrast, Hurtgen (1975), Hurtgen and Whitmore (1978) and Hurtgen and Ganjam (1979) were able to shorten dioestrus in mares by simply dilating the cervix manually. Moreover, Berglund et al. (1982) obtained elevated PGF2 α concentrations in uterine flushing media if flushing was performed transcervically compared to the hysterectomy technique. However, Wilde et al. (1989) could not stimulate any changes of oestrous

cycle and endocrine releasing patterns by placing an external sheath from an embryo transfer catheter into the cervical canal and the caudal part of the corpus uteri for 60 seconds.

To evaluate the effects of cervical manipulation in mares, we developed a standardized method for dilation of the cervix (Handler et al. 2000; 2003). A modified balloon catheter was inserted into the caudal part of the cervical canal via a tulip-formed insertion aid. Fixation of the catheter within the insertion-aid allowed inflating the balloon in the cervical canal without irritation of the endometrium. Dilation of the cervical canal to the maximum diameter of 4.5 cm was performed in each case for ten minutes. The effects of standardized manipulation of the equine cervix were investigated by six clinical studies in cyclic and early pregnant mares at Day 7 of dioestrus and pregnancy, respectively.

Cyclic mares

In cyclic mares, dilation of the cervix at Day 7 (ovulation = Day 0) shortened the length of dioestrus and oestrous cycle significantly by almost two days compared with controls and insertion of the catheter without dilation. Duration of oestrus remained unaltered by manipulation of the cervix (Handler et al. 2000, 2003). In agreement with the oestrous cycle patterns, plasma concentrations of progesterone were significantly lower at Days 9 to 12 and decreased two days earlier to basal levels in dilated mares compared with controls (Handler et al. 2000, 2003). Interestingly, cervical dilation had no effect on plasma PGF2 α -metabolite (PGFM) concentrations, but distension of the vagina through a speculum provoked a slight rise of PGFM in individual mares (Handler et al. 2003). From these findings it can be concluded that the shortening of dioestrus was not caused by direct PGF2 α secretion and subsequent luteolysis. In this case, onset of oestrus would have started much earlier compared with dilation group (3 to 5 vs. 10 days) (Douglas and Ginther 1975, Handler et al. 2004, Oxender et al. 1975). Similarly, luteolysis due to inflammatory response of the endometrium as suggested by Lagneaux et al. (1988) cannot provide a good explanation because the regression of the corpus luteum would have started also much earlier. However, no explanation for the shortage of dioestrus after cervical dilation in cyclic mares can be provided at the moment.

A series of exogenous stimuli such as manual manipulation of clitoris, vagina and cervix, intrauterine infusion of PBS and uterine biopsies (Nikolakopoulos et al. 2000, Paccamonti et al. 1999, Sharp et al. 1997) have been shown to induce oxytocin (OT) secretion in mares. In our study (Handler et al. 2003), cervical dilation stimulated distinct secretion of OT up to maximum plasma concentrations (305.2 pg/mL) compared with insertion (125.5 pg/mL) and controls (6.1 pg/mL). Although, OT plays a major role in the initiation of luteolysis, release of OT during dilation of the cervix is not likely to have a direct effect because OT-receptors are down-regulated at Day 7 and up-regulation starts earliest by Day 10 (Stout et al. 1999). Thus, stimulation of PGF2 α release depends from the respective stage of oestrous cycle and maximum PGFM response to uterine biopsy was achieved at Days 12 and 14 in cyclic mares (Sharp et al. 1997). However, blocking the secretion of OT by administration of xylazine hydrochloride

before dilation abolished the effects on dioestrous and oestrous cycle length (unpublished data). Possible contribution of OT or xylazine hydrochloride - presumably via central effects - is not elucidated until now and needs further investigation.

Pregnant mares

In inseminated mares, cervical dilation at Day 7 did not affect pregnancy rate (6 of 8 vs. 8 of 8 mares in control group) and embryonic growth rates (Handler et al. 2006). One mare acquired uterine inflammation and subsequent early embryonic death after dilation of the cervix. In contrast to cyclic mares, plasma progesterone concentrations did not decrease after dilation, with the exception of two mares, which developed endometritis and luteolysis (Handler et al. 2006). Surprisingly, pre-treatment of the mares with xylazine hydrochloride resulted in enhanced embryonic growth rates in dilated mares compared with controls (Handler et al. 2002, Handler 2005). All mares (n=7) of dilation and 5 of 7 control mares became pregnant after insemination with raw semen from a fertile pony stallion (Handler et al. 2002). Mares treated with xylazine hydrochloride before dilation yielded conflicting plasma progesterone concentration patterns: Mares carrying twins showed lower concentrations in dilation group, mares carrying singletons exhibited no differences among treatment groups (Handler 2005). This observation disagrees with the data in cyclic mares, because significant decrease of progesterone secretion due to cervical dilation was abolished by administration of xylazine hydrochloride (Handler et al. 2003). Although there is no satisfactory explanation available at the moment, the observed phenomenon indicates differences of the endocrine system in cyclic and pregnant mares.

The neuropeptide OT and OT-receptor system play a major role in the initiation of luteolysis in mares similar to other species (McCracken et al. 1999). During early pregnancy the up-regulation of OT-receptors has to be blocked by a signal secreted by the conceptus. While in ruminants, interferon- τ was identified to act as a signal (Lammung et al. 1995), the nature of the respective signal is still unknown. However, in vivo (Goff et al. 1987, Sharp et al. 1997, Starbuck et al. 1998) and in vitro studies (Franklin et al. 1989) clearly demonstrated that the stimulatory effect of OT on the secretion of prostaglandin is markedly reduced or abolished during early pregnancy in mares. The inhibition of PGF2 α release was not associated with suppression of OT secretion or a decrease in OT-receptor density (Sharp et al. 1997).

Based on these facts, we investigated the influence of OT on early embryonic development in mares (Handler et al. 2006). Single administration of oxytocin (10 IU per mare intravenously) or its long-acting analogue carbetocin (280 μ g per mare intramuscularly) at Day 7 had no influence on pregnancy rates and embryonic growth until Day 13. However, the application of xylazine hydrochloride inhibited the secretion of oxytocin during cervical dilation and conceptus growth rates increased (Handler 2005). Unfortunately, explanation for these apparent contradictory data and possible mechanisms responsible cannot be given at the moment.

At least, responses of the secretion of the anterior pituitary hormones LH and prolactin to dilation of the cervix have been

studied. Both glycoproteins did not show changes of secretion patterns due to cervical manipulation (unpublished data), with the exception of one mare, which developed uterine inflammation and subsequent early embryonic death by Day 12 after cervical dilation. The concerning mare exhibited a distinct rise of plasma prolactin from basal (3.6 µg/L) to maximum concentrations (68.2 µg/L) immediately after dilation. Prolactin in non-lactating mares has been demonstrated to undergo an annual secretion pattern (Roser et al. 1984), but no changes of concentrations were detected during the oestrous cycle (Johnson 1986). However, possible functions of prolactin during the oestrous cycle have not been elucidated by now. Thus, no sufficient explanation of the mechanisms responsible for the distinct rise of prolactin after cervical dilation and its impact on successive uterine inflammation and embryonic death can be given at the moment.

Conclusion

Cervical dilation has been demonstrated to have no negative impact on pregnancy rates and early embryonic development in mares. Thus, irritation of the cervix itself does not seem to contribute to variable pregnancy rates after transcervical transfer of embryos. Moreover, data indicate that other manipulations, e.g. surgery of the caudal cervix are not likely to affect the early pregnancy, provided that it will be performed without any irritation and bacterial contamination of the uterine lumen.

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