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Hormonal diagnosis of equine cryptorchidism

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Schlüsselwörter: Hengst, Kastration, Östrogene, Testosteron, Blut, Kot, Urin.

Summary

For the hormonal diagnosis of cryptorchidism in horses measurements of testosterone and oestrogens are used. For this purpose the determination in blood and, for oestrogens, also in faeces is suited. The aim of this study was to determine the decrease of steroid hormone levels in blood and faeces of stallions after castration to evaluate the time lag until levels of geldings are reached. In addition, the usefulness of measuring urinary oestrogens for diagnosis of cryptorchids was evaluated. Mature stallions (n = 38) and cryptorchids (n = 5) of various breeds were castrated. Blood samples were taken prior to castration and 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after removing the second testis. Daily faecal samples were collected for 14 days and on day 21 after castration. Large individual differences were found concerning both maximal concentrations of steroids and the time course of their decrease. Threshold levels for differentiating geldings from horses with testicular tissue were reached in the blood within 0.5 - 24 h (median: 4 h) and 4 - 48 h (median: 12 h) for testosterone and conjugated oestrogens, respectively. For faecal oestrogens this level was reached 1 - 21 days (median: 5 days) after castration. These withdrawal times must be taken into account if steroid measurements are used as a tool to diagnose remaining testicular tissue after castration.

As in the urine of stallions or cryptorchids approximately 1,000 times higher (as compared with geldings) concentrations of conjugated oestrogens were found, it will be possible to discriminate geldings from horses with testicular tissue by measuring urinary oestrogens. The avoidance of a stimulation test and the need of only a single urinary sample is advantageous.

Zusammenfassung

Hormonelle Diagnostik des Kryptorchismus beim Pferd

Zur hormonellen Kryptorchismusdiagnose beim Pferd dient die Messung von Testosteron und Östrogenen. Dafür eignet sich eine Bestimmung sowohl im Blut als auch von Östrogenen im Kot. Das Ziel dieser Studie war es festzustellen, ab welchem Zeitpunkt nach der Kastration ein Pferd hormonanalytisch eindeutig als Wallach identifiziert werden kann. Zusätzlich sollte die Eignung einer Östrogenbestimmung im Harn zur Kryptorchismusdiagnose überprüft werden. Geschlechtsreife Hengste mit physiologisch gelegenen Hoden (n = 38) sowie kryptorche Pferde (n = 5) unterschiedlicher Rasse wurden untersucht. Blutproben wurden vor der Kastration und 0,5; 1; 2; 4; 8; 12; 24; 48 sowie 72 Stunden nach dem Absetzen des 2. Hodens genommen. Eine Sammlung der Kotproben erfolgte täglich 14 Tage lang sowie am Tag 21 nach der Kastration. Große individuelle Unterschiede der Steroidkonzentration konnten sowohl vor der Kastration als auch danach festgestellt werden. Die Testosteronkonzentration im Blut erreichte innerhalb von 0,5 - 24 h (Median: 4 h), die Östrogene innerhalb 4 - 48 h (Median: 12 h) nach der Kastration Werte, die eine sichere Unterscheidung zwischen Wallachen und Pferden mit hormonaktivem Hodengewebe ermöglichen. Im Kot wurden diese Werte erst zwischen dem 1. und 21. Tag (Median: 5. Tag) erreicht. Dieser Zeitraum muß berücksichtigt werden, wenn Steroidhormonbestimmungen zur Diagnose von eventuell verbliebenem Hodengewebe nach einer Kastration eingesetzt werden sollen.

Da im Vergleich zu Wallachen im Harn von Hengsten bzw. Kryptorchiden 1 000fach höhere Östronsulfatkonzentrationen gemessen wurden, ist eine Bestimmung von Östrogenen im Harn zur Kryptorchidendiagnostik geeignet. Von Vorteil ist dabei, daß auf einen Stimulationstest verzichtet werden kann und zur Bestimmung eine einzige Harnprobe ausreicht.

Introduction

Horses, which have no visible or palpable testes but behave in some way like stallions are a recurrent problem, especially if they are presented with a previous history of castration. As a significant number of such horses are geldings (COX, 1986; LINE et al., 1985), laboratory tests were developed to eliminate unnecessary surgery. In the stallion, testosterone concentrations show large diurnal and seasonal variations (COX et al., 1973; RAESIDE, 1978). Therefore the measurement of testosterone prior to and following stimulation with human Chorionic Gonadotropin (hCG), which was first proposed by COX et al. (1973), is used to determine the presence of testicular tissue in horses. In addition, large amounts of oestrogens are produced by the equine testis (SETCHELL and COX, 1982), a small part of which is excreted via the faeces. Their

measurement both in blood and faeces also proved suited for the diagnosis of cryptorchidism (ARIGHI and BOSU, 1989; COX et al., 1986; PALME et al., 1994).

The aim of this study was to determine the decline of steroid hormone concentrations in blood and faeces of stallions after castration to evaluate the time lag until levels of geldings are reached thus allowing the determination as to whether all testicular tissue was successfully removed during surgery. In addition, as oestrogens in the horse are almost totally (98 %) excreted via the urine (PALME et al., 1996), urinary oestrogens were measured to test the suitability of their determination for diagnosing equine cryptorchidism.

Material and Methods

In a first experiment mature stallions (n = 38) and cryptorchids (n = 5) of various breeds were castrated. Blood samples were taken prior to castration and 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after removing the second testis. Daily faecal samples were collected for 14 days and on day 21 after castration. Concentrations of testosterone and conjugated oestrogens (oestrone sulphate) in blood plasma and unconjugated oestrogens in the faeces were measured as previously described (PALME et al., 1994).

In a second experiment urine was collected from 20 stallions (8 Haflinger and 12 Thoroughbred horses) every third week from November until the end of May. These stallions were 6 to 19 years old and 10 of them were used from March to May once a week for semen collection. In addition, urinary samples were taken from geldings (n = 22), stallions (n = 20) and cryptorchids (n = 8; confirmed by surgery) of various breeds at different times during the year. Conjugated oestrogens in urine were determined similar to those in blood (PALME et al., 1994).

Statistical analysis (Kolmogorov-Smirnov test and Mann-Whitney U-test) was done using SigmaStat - software package (SPSS ASC GmbH, Germany). As the concentrations of the steroids were not normally distributed, figures are shown as boxplot diagrams (Figs. 1 - 4).

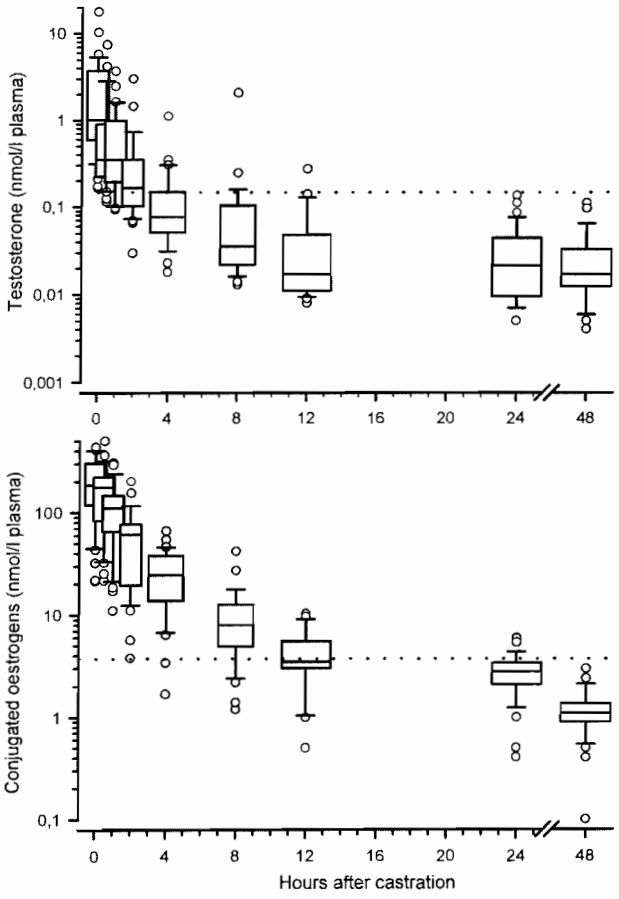

Fig. 1: Boxplot of plasma concentrations (nmol/l; logarithmic scale) of testosterone (upper panel) and conjugated oestrogens (lower panel) in horses after castration; the dotted lines represent the level (0.14 nmol/l and 3.5 nmol/l, respectively) for differentiating geldings from animals with testicular tissue.

Results

Large individual differences were found concerning both maximal concentrations of steroids and the time course of their decrease. Before castration, testosterone values ranged from 0.18 nmol/l to 17 nmol/l plasma (median: 1 nmol/l), conjugated oestrogens varied between 21.5 nmol/l and 504 nmol/l plasma (median: 195 nmol/l) and faecal oestrogens between 42 nmol/kg and 585 nmol/kg faeces (median: 175 nmol/kg). Although steroid hormone concentrations were still decreasing, threshold levels for differentiating geldings from horses with testicular tissue (testosterone: < 0.14 nmol/l; conjugated oestrogens: < 3.5 nmol/l; faecal oestrogens: < 38 nmol/kg; PALME et al., 1994) were reached in the blood within 0.5 - 24 h (median: 4 h) and 4 - 48 h (median: 12 h), respectively (Fig. 1). For faecal oestrogens this level was reached 1 - 21 days (median: 5 days) after castration (Fig. 2).

In the 20 stallions concentrations of conjugated oestrogens in urine tended to increase during the sampling period from November until May (Fig. 3). However differences were significant ($p<0.05$) in only a few instances of sampling

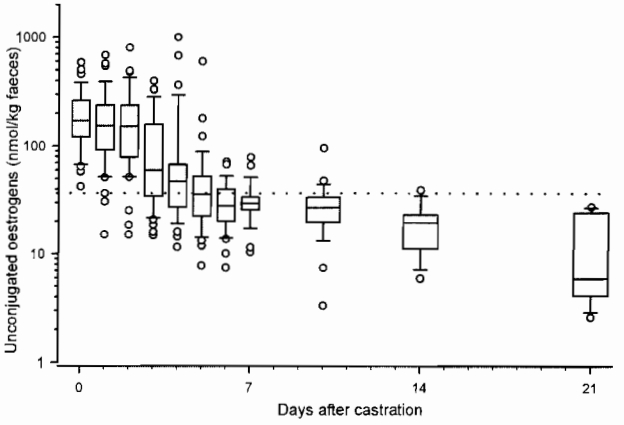

Fig. 2: Boxplot of unconjugated oestrogen concentrations (nmol/kg; logarithmic scale) in the faeces of horses following castration; the dotted line represents the level (38 nmol/kg) for differentiating geldings from animals with testicular tissue.

(Fig. 3). Urinary oestrogen concentrations of geldings ranged from 0.6 to 33.3 nmol/l (median: 11.3). In stallions (total of 220 samples) and cryptorchids approximately 2,000 to 10,000 times higher concentrations were observed. They ranged from 1,500 to 306,000 nmol/l (median: 66,000) and 1,900 to 348,000 nmol/l (median 14,500), respectively (Fig. 4).

Discussion

To our knowledge the time course of the decline of testosterone and oestrogen concentrations has not been determined previously in horses after castration. Following castration the steroids were quickly eliminated from the blood as it was seen after infusion of radiolabelled steroids (PALME et al., 1996). In all horses within 2 days testosterone and oestrogen concentrations reached threshold levels (PALME et al., 1994) for differentiating geldings from horses with testicular tissues. For oestrogens in the faeces this level was reached later, as they are excreted via the bile into the gut and transported with the digesta. These withdrawal times must be taken into account if steroid measurements are used as a tool to diagnose remaining testicular tissue after castration of abdominal cryptorchids. Such an early proof of the removal of abnormal located, steroid producing tissue might be useful in some cases.

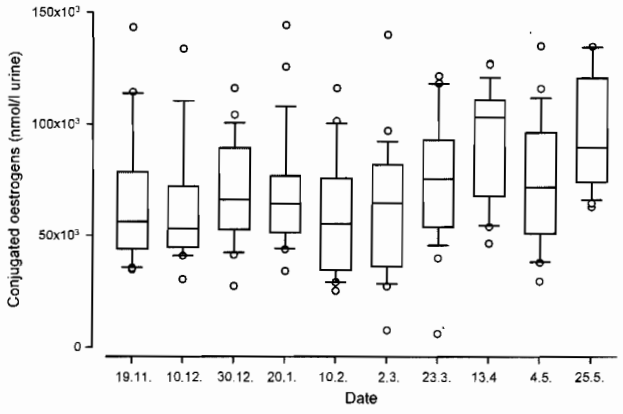

Fig. 3: Boxplot of conjugated oestrogen concentrations (nmol/l) in the urine of 20 stallions from November until May; concentrations were significantly higher ($p<0.05$) on days 25.5. (vs. 10.12., 10.2. and 2.3.) and 13.4. (vs. 10.12. and 10.2.).

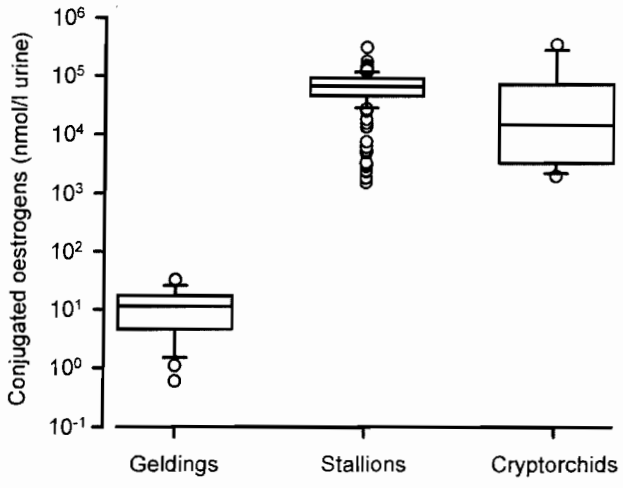

Fig. 4: Boxplot of urinary conjugated oestrogens (nmol/l) in geldings, stallions and cryptorchids (note the logarithmic scale!)

In the horse, oestrogens are almost totally excreted via the urine (PALME et al., 1996) and high amounts in the urine have been reported in the stallion (PIGON et al., 1961). Therefore urinary samples of stallions, cryptorchids and geldings were analysed to evaluate the suitability of such determinations for a diagnosis of cryptorchidism. The high concentrations of urinary oestrogens in stallions in our study are in accordance with previous findings (PIGON et al., 1961). Variations in the oestrogen content of the urine in individual stallions during the sampling period were found exhibiting a tendency towards higher levels during spring. Similar results were reported by RAESIDE (1978) for plasma oestrogens. However, as concentrations in the urine of stallions and cryptorchids are approximately 1,000 times higher in relation to geldings, seasonal variations do not diminish the usefulness of an analysis of urinary oestrogens for diagnosis of equine cryptorchidism. As in blood and faeces (PALME et al., 1994), oestrogen concentrations in the urine of cryptorchids were similar to those of stallions.

Hormonal diagnosis of equine cryptorchidism is successfully achieved by measuring sex-steroids in blood and faeces (ARIGHI and BOSU, 1989; COX et al., 1986; PALME et al., 1994). Our study suggests that measuring urinary oestrogens offers a useful, non-invasive method for the diagnosis of cryptorchidism in the horse. As some kinds of plastic may absorb steroids, urine (only a few ml are necessary) should be collected in glass vials. One must be aware of the fact that the presence of testicular tissue can not be diagnosed hormonally in immature (younger than 2 to 3 years) horses (ARIGHI and BOSU, 1989; COX et al., 1986; PALME et al., 1994). Extremely high oestrogen concentrations (in relation to low values in geldings) in urine are especially advantageous as only a single sample is needed and a stimulation test can be avoided.

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