Genetic study of cryptorchidism in Swedish Icelandic and Standardbred horses

Kim Jäderkvist
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Genetisk studie av kryptorkism hos svenska Islandshästar och Varmblodstravare

Kim Jäderkvist

Supervisors:
Gabriella Lindgren, SLU, Department of Animal Breeding and Genetics
Susanne Eriksson, SLU, Department of Animal Breeding and Genetics
Jeanette Axelsson, SLU, Department of Animal Breeding and Genetics

Supportive supervisor:
Anne-Marie Dalin, SLU, Department of Clinical Science

Examiner:
Anna Näsholm, SLU, Department of Animal Breeding and Genetics

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1. Sammanfattning

Kryptorkism är en defekt som drabbar hanar och innebär att en testikel (unilateral) eller båda testiklarna (bilateral) inte vandrar ner i pungen som normalt. Det är en medfödd defekt och förekommer hos flera olika arter, bland annat nötkreatur, grisar, får, hästar, hundar och människor. Hos hästar finns uppgifter om att mellan 2-8 % av hingstarna är klassade som kryptorkida, beroende på ras. Arvbarheten för kryptorkism har skattats för grisar och hundar men vi har inte hittat någon studie gjord på häst. I den här studien skickades en enkät ut till svenska uppfostrare av varmblodiga travhästar och islandshästar för att samlas in information om när testiklarna hos hingstarna har vandrat ner i pungen. Varmloden definierades som kryptorkida om de inte hade fått ner testiklarna i pungen vid sex månaders ålder medan gränsen för islandshästarna sattes till tolv månader. Informationen från varmloden analyserades inte vidare då det var för få uppfostrare som hade information om testikelnedvandringen. För islandshästarna svarade 56,6 % av uppfostrarna som fått enkäten, vilket gav information om 858 hästar. Förekomsten av kryptorkism påverkades signifikant av avelsvärde för mankhöjd, gård samt födelseår. Genetiska parametrar för om hästen saknade någon testikel i pungen vid olika åldrar estimerades och arvbarheten skattades till mellan 0,1 och 0,3 på den synliga skalan (0/1), vilket motsvarar mellan 0,3 och 0,5 på den underliggande kontinuerliga skalan.

2. Abstract

Cryptorchidism is a defect affecting males and means that one testis (unilateral) or both of the testes (bilateral) fail to descend normally into the scrotum. It is an indigenous defect and it exists in several species, for example cattle, pigs, sheep, horses, dogs and humans. In horses there is information that about 2-8 % of the stallions are classified as cryptorchid, depending on breed. The heritability for cryptorchidism has been estimated for pigs and dogs but we have not found any studies done on horses. In this study a questionnaire was sent out to Swedish breeders of Standardbred horses and Icelandic horses to collect information about when the testes of the stallions had descended into the scrotum. Standardbred horses were defined as cryptorchid if they had not had the testes down at six months of age and for the Icelandic horses twelve months was set as limit. The information from the Standardbred breeders was not used for further analysis since there were too few breeders that knew anything about their horses’ testis descent. For the Icelandic horses 56,6 % of the breeders answered the questionnaire which gave information for 858 horses. The incidence of cryptorchidism was significantly affected by breeding value for height at withers, farm and birth year. Genetic parameters for if the horse missed one or both testes at different ages was estimated and the heritability was estimated to between 0.1 and 0.3 on the visible scale (0/1) which corresponds to between 0.3 and 0.5 on the underlying continuous scale.

3. Introduction

3.1 Background

Cryptorchidism, which means hidden testis in Greek (Klonisch et al., 2004), is a defect affecting males and means that one testis (unilateral) or both of the testes (bilateral) fail to descend normally into the scrotum. Normally this happens before or shortly after birth (Bergin et al., 1970; Leipold et al., 1986; Lu, 2005). Bergin et al. (1970) found that the earliest descent of both testes was in a 315 days old fetus. Usually the testes in horses descend into the scrotum between 30 days prior to and two weeks after birth (Lu, 2005) and the internal inguinal ring closes during the first two weeks after birth (Pickett et al., 1989). Cryptorchidism is an indigenous defect and it exists in several species, for example cattle,
pigs, sheep, horses, dogs, cats, rabbits and humans (reviewed by Amann & Veeramachaneni, 2007).

Cryptorchidism causes problems for the animal and also for the owner or the farmer, because of reduced sperm production of the male (Blanchard et al., 2006) or even sterility (Mueller & Parks, 1999; Bladon, 2002), increased risk for tumors (Amann & Veeramachaneni, 2006), more complicated castration procedures and increased costs. So it is important to try to find the background for the defect and estimating heritability is a first step in this process.

3.2 Aim of the study
The aim of this study was to collect information about testis descent for individual horses through a questionnaire sent to Swedish breeders of Icelandic and Standardbred horses, and use the information to estimate genetic parameters for cryptorchidism in horses. To our knowledge, this has not been done before.

Calculating the heritability is one step in the process of studying the genetic background and the possibility to breed against the defect.

4. Literature review

4.1 Cryptorchidism in horses
Cryptorchidism is said to be a complex condition influenced by genetic, epigenetic and environmental factors (Amann & Veeramachaneni, 2006). The time of descent of the testes can vary much, in some horses the inguinal testes can descend into the scrotum up to four years of age (Cox et al., 1979). Horses that are cryptorchid are often called ridglings or rigs (Bladon, 2002). The time of testis descent has been seen to vary a lot between breeds and also between individuals. For example Icelandic horses are normally later than the Standardbred trotter (Axelsson, J., pers. comm. 2012).

In horses, depending on breed, 2-8 % of the stallions are reported to be cryptorchid (reviewed by Amann & Veeramachaneni, 2007). In a study by Hayes (1986) they had data from 16 North American veterinary medical teaching hospitals for almost 59 000 colts and stallions of different breeds and different ages. About eight percent, or just over 5000 horses, missed one or both testes. However, it is hard to know how many cryptorchid stallions that remain cryptorchid throughout their lives, some of them might get their testes down when they are quite old and a small fraction might be castrated before the time of descent (Leipold et al., 1986). Hayes (1986) found that some breeds had a significantly higher risk for getting the defect, such as; Percheron, American saddle horse, American Quarter horse, ponies and crossbred horses. In a study by Engsäll (2011) it was indicated that Icelandic horses seems to have a comparatively high incidence of cryptorchidism. Out of 164 castrated Icelandic stallions (castrated at four different animal hospitals in Sweden during a ten year period, 2000-2009), 43 % were cryptorchid. In the same study it was found that the proportion of cryptorchid Swedish Standardbred horses was 8 % of 625 horses. In Sweden there are about 1000 Icelandic horses (SIF, 2012) and 4000 Standardbred horses (ST, 2012a) born every year. If 50 % of them are males then the proportion of horses castrated at one of the veterinary clinics in the study was about 3 % for both breeds (164/5000 respectively 625/20 000).

4.2 Monorchidism
Another condition where one of the testes is absent from the scrotum is monorchidism. Cryptorchidism is when one or both testes are retained somewhere along the path of normal
descent while in monorchidism there is a complete absence of one testis, no matter if it is due to agenesis or degeneration of the testis. If the agenesis is bilateral (affecting both of the testes) the stallion will develop phenotypically female external genitalia. The diagnostic and surgical approaches for monorchid and cryptorchid horses are similar. Monorchidism is rare in horses (Parks et al., 1989). Even if a horse have had its testes down early in life, it is possible that he pulls them up in the inguinal canal again, later in life. The horse is then said to have retracted testes (Amann & Veeramachaneni, 2006).

4.3 Heritability of cryptorchidism
Equine cryptorchidism has long been said to be heritable (for example Hayes, 1986; Leipold, 1986), but no one has estimated the heritability for cryptorchidism in horses. It has been done for other species, however. For example in dogs the heritability has been estimated to 0.23 using a threshold model in one study (Nielen et al., 2001) and, in another study, between 0.46 and 0.75 on the underlying scale, for different breeds (Dolf et al., 2010). The higher heritability in the latter could be explained by a different definition and evaluation of the phenotype, for instance that they only investigated abdominal cryptorchidism. For pigs, the heritability of liability to cryptorchidism has been estimated to about 0.5 (Mikami & Fredeen, 1979) and in other studies the heritability has been estimated to between 0.02 and 0.7 (reviewed by Mattson, 2011). In the study by Mattson (2011) the heritability on the visible scale was estimated to 0.005-0.23 for different breeds and if the estimations were based on information on boars as fathers or maternal grandfathers of the litters. Three different breeds were studied using information from 15 nucleus herds; Hampshire, Yorkshire and Landrace. There were large breed differences; the heritability for Yorkshire was higher compared to Hampshire and Landrace. The difference between the estimations based on information on boars as fathers or grandfathers was not that large and for Yorkshire the heritability was higher when using the information on boars as grandfathers while for Hampshire and Landrace the opposite was found (Mattson, 2011).

4.4 Terminology & anatomy
4.4.1 Development of the testes
The testes in the male horse are developed quite early during gestation. After about five weeks of gestation, a recognizable testis is developed. The gubernaculum of the testes, which is a ligament that contributes to the descent of the testes, develops just after; it can be recognized in the male fetus at about six and a half weeks of gestation. When the estrogen levels in the mare increases, the number of Leydig cells in the fetus increases and this leads to an enlargement of the testes. This enlargement continues until the 8th month of pregnancy. After that, also due to the hormonal changes in the mare, the size is decreased (Leipold et al., 1986). Normally the testes pass from the abdomen to the scrotum via the inguinal canal (Mueller & Parks, 1999). This movement usually happen between the 9th and 11th month of gestation (Leipold et al., 1986) and the overall process is divided into three parts; abdominal testis translocation, transinguinal migration of testis and inguinoscrotal migration of the testis (Amann & Veeramachaneni, 2006; Amann & Veeramachaneni, 2007). Due to maternal endocrine stimulation, the gubernaculum is shrinking, and the testes are pulled into and through the inguinal canal. There is also an intra-abdominal pressure which contributes to the movement of the testes. The right testis is smaller than the left and might sometimes slip back into the inguinal canal (Leipold et al., 1986). The first weeks after birth the gubernaculum is quite large and it might be mistaken for a testis (Bergin et al., 1970). Each of the testes weigh between 5-20 g at birth and the increase in size is quite slow during the first ten months.
Normally, after 12-18 months of age a more rapid development of the testes starts, but this time could vary a lot between individuals (Amann, 2011).

### 4.4.2 Undescended testes

In a cryptorchid horse the undescended testes can be found in three different locations; the abdominal cavity, the inguinal canal or subcutaneous, i.e. under the skin (Amann & Veeramachaneni, 2006). The testis can also be found partly in the abdomen; if the testis is located in the abdomen, while some amount of spermatic cord and the tail of epididymis are extended into the inguinal canal, it is called partial abdominal cryptorchidism (Genetzky et al., 1984). If the testis is found in the abdominal cavity, it indicates that it was a problem to initiate and complete the abdominal translocation of the testis. Most often the testis is located between the kidney and the bladder, or near the inguinal ring. If the testis instead is inguinal this indicates problems with the transinguinal migration of the testis and the testis is located in the space between the internal and the external inguinal rings (Amann & Veeramachaneni, 2006). A stallion with an inguinal testis is also called a “high flanker” (Leipold et al., 1986). If the testis is located subcutaneously, this reflects failure in the inguinoscrotal migration of a cauda epididymis and testis into the scrotum. The testis then lies under the skin outside the abdominal wall (Amann & Veeramachaneni, 2006). In horses subcutaneous testes are uncommon (Amann & Veeramachaneni, 2007). It is categorized as a cryptorchid testis but sometimes it is referred to as an ectopic testis (Genetzky, 1984). A subcutaneous testis can be palpated externally but it is usually not possible to identify a testis in the inguinal canal in the same way (Stickle & Fessler, 1978). Figure 1 shows an overview of the stallion reproductive tract.

![Figure 1](http://www.hastsverige.se/sida304.html) 

**Figure 1.** The stallion reproductive tract. Modified from [http://www.hastsverige.se/sida304.html](http://www.hastsverige.se/sida304.html), from Björn Sandgren: Hästens Anatomi, Svensk Travsport.

In the cryptorchid horse, both testes are not always located at the same place (Amann & Veeramachaneni, 2007) and if the left testis is retained it is more often abdominal while the right testis have been found to be more inguinal (Stickles & Fessler, 1978; Cox et al., 1979; Coryn et al., 1981). This is different from pony breeds where inguinal cryptorchidism is more common on the left side and the right testis is more often located in the abdomen (Cox et al., 1979; Coryn et al., 1981). It is more common that the left testis is retained, this might be because of the size difference, the left testis is often larger than the right (Leipold et al., 1986). The fact that the left testis more often is located in the abdomen compared to the right testis
might be due to the larger size and a slower descent (reviewed by Mueller & Parks, 1999). Bergin et al. (1970) reported that in 78 % of 32 fetuses between nine month of gestation and birth the right testis had descended further down than the left one. Only in 3 % of the fetuses the left testis had descended further than the right (Bergin et al., 1970).

Coryn et al. (1981) found that among 205 cryptorchid horses of various breeds, over 90 % were affected unilaterally and only about 9 % was bilateral. In 67 % of the unilateral horses, the testis was located in the inguinal canal. That unilateral is more common than bilateral cryptorchidism agree with findings reviewed by Amann & Veeramachaneni (2007). Two studies have shown that the ratio of inguinal cryptorchidism versus abdominal cryptorchidism decreases with age of the horse (Cox et al., 1979; Coryn et al., 1981). According to Cox et al. (1979) the reason is not only that most stallions are castrated when they are young, it can also be due to that in some horses the inguinal testes descent into the scrotum up to four years of age. This means that there will be few older horses that are inguinal cryptorchid (Cox et al., 1979).

4.5 Aetiology
The causes of cryptorchidism are obscure and the incomplete testis descent is a complex process that is not completely understood. The process involves genetic, hormonal and mechanical factors (Hayes, 1986). Today it is generally accepted that cryptorchidism is due to many causes (Leipold et al., 1986; Amann & Veeramachaneni, 2006). There are more than 20 genes associated with human cryptorchidism (Klonisch et al., 2004). According to Bergin et al. (1970) there are four obvious factors for abdominal cryptorchidism; insufficient stretching of the ligament gubernaculum, insufficient growth of the gubernaculum leading to an insufficient expanding of the inguinal canal, insufficient abdominal pressure and also that the testis is not located in the right place when it is exposed to the pressure (Bergin et al., 1970).

In the study by Hayes (1986) crossbred horses as a group appeared to be at a higher risk for cryptorchidism compared to purebreds of several breeds. It was suggested that other factors than genetics also are involved in equine cryptorchidism and one factor could possibly be the endogenous hormonal state of the pregnant female (Hayes, 1986). For equine cryptorchidism, both autosomal dominant and autosomal recessive inheritance patterns have been suggested (Leipold et al., 1986). In one study it was found that in the equine cryptorchid testes, the INSL3 protein was significantly reduced. INSL3 is a peptide hormone, necessary for the differentiation process of the ligament gubernaculum testis, but it is still not known what potential physiological role INSL3 has in cryptorchidism (Klonisch et al., 2003). On male mice it has been reported that a deletion of the Ins3 gene gives impaired testicular descent and infertility (Nef & Parada, 1999). In pigs there are several genes, including INSL3, that are known to be involved in the development of cryptorchidism, for example CGRP and HGF (reviewed by Zhao, 2009). Since cryptorchidism probably is hereditary, breeding with unilateral cryptorchid stallions should be avoided (Leipold et al., 1986; Amann & Veeramachaneni, 2006).

4.6 Diagnosis of cryptorchid horses and false rigs
Most stallions that are not used for breeding are castrated. However there are problems with male horses that do not have any visible or palpable testes, but still show stallion-like behaviour. These horses are called “phantom” stallions. It can be horses that are bilateral cryptorchids, hemi-castrates or geldings showing stallion-behaviour, a so-called false rig (Palme et al., 1994).
A hemi-castrated stallion is a horse where only one testis is removed during the castration i.e. when the contemplated castration procedure fails, which is a quite common problem for the equine practitioner (Mueller & Parks, 1999). The unilateral castration is an elective procedure (Maxwell, 2005).

4.6.1 Measuring estrogen concentration
Several studies have investigated methods for detecting cryptorchid horses (Cox et al., 1973; Arighi et al., 1986; Cox et al., 1986; Arighi & Bosu, 1989; Cox, 1989; Silberzahn et al., 1989; Palme et al., 1994). One way to diagnose a cryptorchid horse is to measure the estrogen concentration in the faeces. Estrogen is a metabolite from testosterone and retained testes will in most cases produce enough testosterone to give detectable estrogen levels in faeces. It has been found that this method is quite accurate and it is a useful tool for diagnosing the horse without using invasive methods (Palme et al., 1994). It is also possible to measure conjugated plasma estrogen (Cox et al., 1986) or the estrone sulphate concentration (Arighi et al., 1986; Arighi & Bosu, 1989). Measuring the conjugated plasma estrogen or the estrogen sulphate concentration are useful methods to differentiate geldings from horses with any testicular tissue but the methods have lower accuracy for horses younger than three years (Arighi et al., 1986; Cox et al., 1986; Arighi & Bosu, 1989). Normally younger horses have lower levels of testosterone and estrone sulphate and for them it is recommended to do more than one test (Arighi et al., 1986).

4.6.2 Impact of human chorionic gonadotropin
In several studies the impact of human chorionic gonadotropin (HCG) on estrogen (Arighi & Bosu, 1989; Silberzahn et al., 1989) and testosterone concentration (Cox et al., 1973; Arighi & Bosu, 1989; Cox, 1989; Silberzahn et al., 1989) has been studied. Increases in testosterone concentration after HCG injection indicates that there is testicular tissue present (Arighi et al., 1986). After the injection of HCG the testosterone levels in both cryptorchid horses and normal stallions raised (Cox et al., 1973; Arighi & Bosu, 1989; Cox, 1989; Silberzahn et al., 1989) but the response in cryptorchid horses was in some studies somewhat poorer (Cox, 1989; Silberzahn et al., 1989). HCG also stimulated an increase in estrogen in both normal stallions and cryptorchid horses but the increase was lower for the cryptorchid horses (Arighi & Bosu, 1989; Silberzahn et al., 1989). In geldings the HCG injection did not affect the plasma steroid levels (Cox et al., 1973; Arighi & Bosu, 1989; Silberzahn et al., 1989). The same thing was found in false rigs, the testosterone levels were comparable to those of the geldings. The behaviour of a false rig is not associated to elevated testosterone levels and it support the idea of that they do not have any extra-testicular source of androgens. Instead their masculine behaviour might have been developed before castration and persisted despite the absence of androgen (Cox et al., 1973).

Identifying a false rig by injecting HCG and then measure the testosterone levels (Cox et al., 1973) cannot be used reliably on animals younger than 18 months since young stallions normally have lower testosterone concentrations than older ones. The testosterone levels and response to HCG is also affected by season and the test during the winter might be of limited application (Cox, 1975).

4.6.3 Ultrasonography
One method for determining the location of the testes is to use ultrasonography. Schambourg (2006) found that ultrasonography, when used during optimal conditions, will locate the retained testes, both in the abdomen and inguinal canal, in 97.5 % of all cases. The technique
is reported to be easily applied to a field situation and a safe, sensitive and specific tool that can be used in all horses regardless of size (Schambourg, 2006).

Before establishing a diagnosis for cryptorchidism by examining the horse clinically, profound sedation of the horse is in most cases necessary (Bladon, 2002).

4.7 Effects of cryptorchidism on reproduction
In most cases unilateral cryptorchid stallions are fertile if they have one normal testis, but bilateral horses are sterile (Mueller & Parks, 1999; Bladon, 2002). In cryptorchid horses the development of the tubular epithelium of the retained testis is not complete and it is incapable of producing sperm (Arighi et al., 1986). But even if they are not sperm producing, it is not recommended to keep a cryptorchid stallion since the testes still produces testosterone as in a normal stallion (Coryn et al., 1981), because the Leydig cells (producing for example testosterone) in the cryptorchid testes are normal (Leipold et al., 1986). The Leydig cells have a better ability to stand the temperature in the stallion’s body, compared to cells of the seminiferous epithelium (Arighi & Bosu, 1989). This means that the behavioural phenotype will be the same as for an intact stallion. Therefore many cryptorchid stallions are castrated (Bladon, 2002). Late descended testes might also cause testicular hypoplasia in some horses (Cox et al., 1979). Blanchard et al. (2006) reported a decrease in both sperm and hormone production in stallions with thermally induced testicular degeneration.

Estrogens are necessary for the male reproduction and it has been found that estrogen can affect proliferation, differentiation and cell function of the reproductive system in males. Different cell types in the testis produce estrogen; Leydig cells, Sertoli cells and germ cells among others (Bilinska et al., 2006). In retained testes, the production of estrogen appears to be reduced (Ryan et al., 1986).

4.8 Associations between cryptorchidism and other factors
Cryptorchidism might be correlated with different traits or other defects. In humans it has been found that for example prematurity, low birth weight and maternal exposure to estrogens during the first trimester leads to an increased frequency of cryptorchidism (Klonisch et al., 2004).

Below follows a summary of some of the factors associated with cryptorchidism.

4.8.1 Sex ratio of litters and litter size
In dogs and pigs it has been found that one or two cryptorchid individuals in the litter increases the proportion of male animals. Also the sex difference within the litter, e.g. the number of males minus the number of females, increases with the presence of cryptorchid individuals (Dolf et al., 2008). A similar result was found by Gubbels et al. (2009), dogs were either classified as a “carrier” (if they had had one or several cryptorchid offspring) or “non-carrier” (no cryptorchid offspring). In litters from two carrier parents were an increased proportion of male animals and also an increased litter size compared to litters from two non-carrier parents. When mating a carrier with a non-carrier these effects was not seen and the litters were not significantly different from the litters from two non-carriers. The proportion of cryptorchid individuals was 2.1 % in the whole population while in the group with two carrier parents the proportion was 24.1 % (Gubbels et al., 2009). The association of cryptorchidism and litter size in pigs was found by Mattson (2011) and Dolf et al. (2008) who found that the total number of born piglets was significantly associated with the prevalence of cryptorchidism.
4.8.2 Associated developmental defects
According to Hayes (1986) most cryptorchid stallions, especially if they are unilateral, are not affected by any other anatomical abnormalities. However if the horse is bilateral, this might indicate a more severe anatomical aberration and it is necessary to do a more careful inspection of the horse. In humans and dogs, cryptorchidism is often linked to other defects, for example inguinal hernia, hypogonadism and hypospadias, but for horses, except for hypogonadism, there are only a few reports that report other defects present with cryptorchidism. In the study by Hayes the medical history of several cryptorchid horses was investigated and the result showed that out of 5009 horses, 134 had any additional developmental defects. The most frequently reported defects were umbilical hernia, inguinal hernia and hypoplastic testis (Hayes, 1986).

4.8.3 Testicular dysgenesis syndrome
In one study it has been found that postpubertal abnormalities associated with cryptorchidism, for example tumors and abnormal development of germ cells, are not caused by the abdominal location of the testis per se, but the abnormal germ cell development might be a result from exposure to chemical pollutions during critical periods of gonadal differentiation (Veeramachaneni, 2006). These abnormalities or defects associated with cryptorchidism are all considered to be part of a testicular dysgenesis syndrome (TDS) and the underlying cause is probably improper development of fetal testis. It is important to remember that also a non-cryptorchid individual can get other TDS-related defects (Amann & Veeramachaneni, 2007).

4.8.4 Coat colours
The KIT-gene that give tobiano horses their pattern is also involved in developmental processes during fetal stage (Mikko, S., pers. comm., 2012) and it has been found that except for white spotting, the KIT-gene also affects germ cell deficiency in mice (ESPCR, 2012). There is also a candidate gene called crsp has been found in cryptorchid mice with white spotting pattern (ESPCR, 2012).

4.8.5 Height at withers
Withers height might have an effect on the frequency of cryptorchidism. It has been found that ponies have a higher risk of getting the defect (Cox et al., 1979; Hayes, 1986). There is also a study on dogs where they have compared different breed groups, for example toy, miniature and standard poodles. They found that the risk of getting the defect was greater in smaller breeds. It might be related to the physical size of the individual or the growth rate of the structures involved (Hayes et al., 1985).

4.9 Other testicular defects
Except for cryptorchidism there are other defects that might affect the testes of the male horse. A short description of some of them follows below.

4.9.1 Changed size and structure of the testes
Usually, a cryptorchid testis is smaller than normal (Leipold et al., 1986; Bøgh et al., 2001) and it is also soft and flaccid (Leipold et al., 1986) but also horses that are not cryptorchid can have different size and structure of their testes. A reduced size of a testis can either be hypoplasia or degeneration of the testes (Oristaglio-Turner, 2007). In the study by Bøgh et al. (2001) scrotal testes had a significant higher weight than inguinal respectively abdominal testes in stallions older than two years. One reason for why cryptorchid testes not increase in size in the growing animal is the higher temperature inside the body (Dalin, A-M., pers. comm., 2012).
Edwards (2008) wrote that in stallions, hypoplasia is most often associated with cryptorchidism but the reason for hypoplasia in descended equine testes remains to be clarified. If the testis is hypoplastic it affects the fertility negatively since there are few germ cells in such testes. In a hypoplastic testis the epididymis is relatively small compared to the testis. This is different from a degenerated testis where the epididymis is relatively large compared to the testis (Edwards, 2008).

There are two different categories of testicular degeneration, one where there is a known insult, for example trauma, high temperature, neoplasia and toxin exposure, and one so called idiopathic, where there is no identifiable underlying reason. The latter results in a steady declining fertility, sometimes it leads to a total infertility of the horse (Oristaglio-Turner, 2007).

### 4.9.2 Rotated testes

In some horses the testes are rotated. Rotations less than 180° are diagnosed by palpation. It may be temporal and come back. If the rotation is more than 270° the horse may get colic. If the torsion is really severe it leads to acute hematocele and hydrocele which is very painful for the stallion (Edwards, 2008).

### 4.9.3 Tumors

Equine tumors are rare (reviewed by Peterson, 1984) and some of them are not malignant. Both cryptorchid and non-cryptorchid stallions can get testicular tumors but the occurrence in cryptorchid horses is more frequent, probably between 4-11 times higher, compared to a non-cryptorchid male (Amann & Veeramachaneni, 2006). When the testes are located inside the body it is probably an increased risk for neoplasia (Bladon, 2002; Lu, 2005).

One reason for the low incidence of testicular tumors is probably that many horses are castrated when they are young (Gelberg & McEntee, 1987).

The testicular tumors can be divided into four categories; teratoma, seminoma, interstitial tumors and sertoli cell tumors (Peterson, 1984). Teratoma is the most commonly reported testicular tumor in horses (reviewed by Peterson, 1984) while sertoli cell tumor is uncommon. Teratomas, seminomas and interstitial cell tumors are associated with cryptorchidism (Edwards, 2008).

### 4.10 Castration

Castration is a very common procedure and it is usually done on stallions not wanted in breeding and for elimination of male behaviour. Castrated stallions, i.e. geldings, are easier and safer to handle (Schumacher, 2006). The surgery procedure for a cryptorchid, especially the abdominal ones, is more difficult and needs to be done at a veterinary clinic and resulting in considerably higher costs for the horse owner.

Another word used for removal of a cryptorchid testis is cryptorchidectomy. There are different methods used for removal of retained testes. The approach is either inguinal, parainguinal, suprapubic paramedian, laparoscopic or a flank approach (Schumacher, 2006). During examination of the horse, if it is not possible to easily grasp both testes, standing castration should be avoided (Bladon, 2002).

### 4.11 Prevention

There is lack of knowledge about which factors that actually affect cryptorchidism. However, since the defect probably is heritable, unilateral horses should not be used for breeding. But
according to Amann & Veeramachaneni (2006) breeders of racehorses uses their valuable horses for breeding even if they only have one testis. They sometimes remove the undescended testis and continue to breed on the horse (Amann & Veeramachaneni, 2006).

Environmental factors are also likely to influence cryptorchidism. To try to decrease the incidence of cryptorchidism Amann & Veeramachaneni (2006) suggests that females should not be exposed to environmental agents that might be estrogenic, anti-androgenic or toxic. If the female come in contact with several different agents of the same type (for example estrogenic agents) in a critical point of fetus development, it might be damaging to the fetal tissue (Amann & Veeramachaneni, 2006; Veeramachaneni, 2007).

4.12 Different breeding recommendations

According to the Swedish Board of Agriculture, cryptorchidism is not a defect that make the animal suffer or that affect its normal behaviour negatively, and it is currently not included in the appendix of disorders to be considered in breeding (SJV, 2009). But many breeding organisations in Sweden have own restrictions for use of cryptorchid horses in breeding since long. For example The Swedish Trotting Association (ST), the Swedish horse board (SH), the Swedish Warmblood Association (ASVH) and the Swedish breeding association of Icelandic horses (SIF) do not allow use of cryptorchid stallions in breeding (SIF, 2011; ASVH, 2012; SH, 2012; ST, 2012b).

4.13 Registration of cryptorchid Standardbred horses in USA and Canada

Compared to Sweden, the registration of cryptorchid Standardbred horses is different in USA and Canada. When a Standardbred foal is registered in the US Trotting Association (USTA) or Standardbred Canada, ridgling is one of the gender options. The other options are mare, spayed mare, stallion or gelding. The registration is most often done before December 31st the year the foal is born (95 % of the horses). But the gender of the horse is continuously updated, so for example a foal registered as a stallion might later in life be registered as gelding (Chunko, A., pers. comm., 2012). This is different from Sweden where nothing is registered about testis descent in the Standardbred breed.

5. Own study

5.1 Materials and methods

5.1.1 Questionnaire to Swedish horse breeders

A questionnaire on testes descent was sent out to Swedish breeders of Standardbred horses and Icelandic horses. One reason for choosing horse breeders and not horse owners for this survey was that it is more likely that the breeders have the knowledge since most horses go through veterinary checks before they are sold.

The questionnaire was sent out to the breeders via mail and it was also put online on the homepage of the Department of Animal Breeding and Genetics, at The Swedish University of Agricultural Science (SLU) (www.hgen.slu.se). Addresses to breeders of Swedish Standardbreds were provided by The Swedish Trotting Association and for breeders of Icelandic horses most addresses were found on the breeders own homepages. Breeders were chosen based on the number of horses that they had bred during the time period 1990 to 2011, and that an updated address could be found. The questionnaire was sent to 100 breeders of Standardbreds and 80 Icelandic horse breeders. There were more breeders of Standardbreds as there are not that many breeders of Icelandic horses that breed several mares annually, some of the breeders just have one or two horses.
Information about the study was spread online and in Swedish horse magazines to inform about the study and to remind breeders to answer the questionnaire. Also two mails were sent to the breeders to remind them to answer. Except for the homepage of the institution, www.hgen.slu.se, information was also published on some Swedish web pages; www.travsport.se, www.hastsverige.se, www.tidningenridsport.se, www.hastmagazinet.com and www.asvt.se. A notice was also published twice in a Swedish magazine for Icelandic horses called “Islandshästen”.

The questionnaire consisted of two parts, for description letter see appendix 1. The appendix is written in Swedish and not translated into English. The first part (see appendix 2) was a list where the breeder should fill in information for all male horses he or she had bred. The name, birth year, and identification number of male horses from each breeder, gathered from the online databases (The Swedish Trotting Association respectively Worldfengur) were pre-filled in to make it easier for the breeders. For the Icelandic horses it was all horses born between 1990 and 2011 while for the Standardbred horses it was horses born 1996 and later. The reason for that was that the breeders of Icelandic horses in most cases had fewer horses than the Standardbred breeders and it was important to get answers from as many horses as possible.

In the first part one question was divided into three parts, with boxes to tick in for every horse. The question was:
“Had the horse both its testes down in the scrotum at: 1). 1 month of age, 2). 6 months of age, 3). 12 months of age”, and the breeder could tick the answer “yes”, “no” or “not known”.

For the Standardbred horses, if the breeder answered NO on question 2 or 3 for any of the horses, he/she was asked to fill in part 2 of the questionnaire. That horse was then defined as a cryptorchid. Part 2 consisted of more detailed questions for the specific horse (see appendix 3). For Icelandic horses, the same procedure was done except for that the stallions were defined cryptorchid only if they had not had their testes down at 12 months of age. The reason for that was that Icelandic horses normally are later in their testis descent compared to Standardbred horses (Axelsson, J., pers. comm., 2012).

5.1.2 Study visit
One study visit was done on a large Standardbred horse stud in Sweden. During the study visit some of the horses were examined by palpation of their testes, and information about the horses was given by the stable manager.

5.1.3 Analysis of Icelandic horse data
All answers were put together in Microsoft Excel and the data analyses were made in the Statistical Analysis Systems; SAS 9.3 (SAS, 2012) and the DMU-software by Madsen and Jensen (2000). Since there were too few answers from Standardbred horse breeders, those results were not included in further statistical analyses.

5.1.3.1 Definition of traits
For the Icelandic horses, many breeders did not check the testes of their horses before two years of age or when it was time to castrate the horse (between 1-5 years of age). Therefore, two extra categories were made; if the horse had its testes down between one and two years of age (24 months) or if the stallion got them down at two years or older (24 months+). Information about horses born 2011 was not included in the categories 24 months and 24
In this paper the following definitions of cryptorchidism at different ages was used: if the horse missed one or both testis at 1 (Crypt1), 6 (Crypt6), 12 (Crypt12), 24 (Crypt24) or 24+ (Crypt24+) months.

5.1.3.2 Factors influencing cryptorchidism

Different factors may influence whether a horse will become cryptorchid or not. In this study we studied the effects of breeding value for height at withers of the horse, country of birth of the parents, coat colour of the horse, foaling age of the dam, birth year of the horse, farm and inbreeding coefficient of the individual and the dam on the probability of cryptorchidism. Icelandic horses can have many different colours, in all sorts of combinations, and in this limited data it was not possible to test the effect of all colours, but three different colours, common in the Icelandic breed, was chosen; silver, grey and pinto and it was also examined if white markings, either on the legs or on the head, had any effect on the frequency of cryptorchidism. Information about breeding value for height at withers, coat colour, white markings, country of birth for the parents and inbreeding coefficients for all horses were taken from the database Worldfengur. The foaling age of the dams was counted out by taking the age of the dam minus the age of the offspring. The breeding value for withers height, birth year and inbreeding coefficients were analyzed both in classes and as regressions.

For estimation of the effects of different factors on the probability of cryptorchidism, logistic regression in proc glimmix in SAS was used (SAS, 2012). The glimmix procedure fits statistical models (generalized linear mixed models) to data also in case of non-normally distributed response, such as for binary (0/1) traits. A logit link function is used and the logit is an underlying continuous criterion upon which linear regression is conducted.

5.1.3.3 Estimation of genetic parameters

Genetic parameters for Crypt6, Crypt12, Crypt24 and Crypt24+ were estimated using the DMU-software by Madsen and Jensen (2000).

Different statistical models were tested, including maternal effect models, sire models and threshold models, but the limited data size and data structure did not allow the use of complex models. In addition, the use of an animal model seemed to best suit the data structure with rather few offspring per sire. Therefore the following linear animal model was used

\[ y_{ijk} = \text{farm}_i + \text{groupyear}_j + \alpha_k + e_{ijk} \]

where \( y_{ijk} \) is the cryptorchidism status of the individual (0 or 1), \( \text{farm}_i \) is the fixed effect of farm where the horse was born, \( \text{groupyear}_j \) is the fixed effect of birth year of the horse, \( \alpha_k \) is the random additive genetic effect of the \( k \)-th horse \((0, \sigma^2_a)\), \( e_{ijk} \) is the random \((0, \sigma^2_e)\) residual effect, \( \text{A} \) is the additive relationship matrix, \( \sigma^2_a \) is the additive genetic variance, \( I \) is the identity matrix and \( \sigma^2_e \) is the residual variance. The birth year of the horses were divided into six different classes; 1990-1993, 1994-1997, 1998-2001, 2002-2005, 2006-2009 and 2010-2011.

Heritabilities on the underlying scale were approximated from the heritabilities on the visible scale using the formula

\[ h^2_{\text{underlying}} = h^2_{\text{observed}} \frac{[p \cdot (1-p)]/z^2}{p \cdot (1-p)/z^2} \]

where \( p \) is the mean proportion in the population and \( z \) is the ordinate on the standardized normal curve that corresponds to a probability \( p \) (Dempster & Lerner, 1949).

Similarly, standard errors of the heritabilities on the underlying scale were estimated using the model
SE (h²) = SE(h²\text{observed}) [p (1-p)]/z² (Roff, 2001).

6. Results

6.1 Swedish Standardbred horses

The answering frequency for the questionnaire to the Standardbred horses was 19 %. In addition to that, one answer was sent in by e-mail. One mail came back because the address was not correct. Of the 20 answers that came in, only 11 had answered the questionnaire, the other ones simply informed that they did not know anything about their horses’ testes descent. The survey resulted in answers for totally 238 Standardbred horses. This gives an average number of 22 horses per breeder. The results are presented in Table 1. In the brackets the percentage is presented, in total respectively when missing values have been excluded.

Table 1. The answers from the Standardbred horse breeders (percentage in brackets).

<table>
<thead>
<tr>
<th>Answer</th>
<th>1 month</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>33 (13.9/97.1)</td>
<td>69 (29/98.5)</td>
<td>99 (41.6/99)</td>
</tr>
<tr>
<td>NO</td>
<td>1 (0.4/2.9)</td>
<td>1 (0.4/1.5)</td>
<td>1 (0.4/1)</td>
</tr>
<tr>
<td>NOT KNOW</td>
<td>156 (65.5)</td>
<td>139 (58.4)</td>
<td>127 (53.4)</td>
</tr>
<tr>
<td>BLANK</td>
<td>48 (20.2)</td>
<td>29 (12.2)</td>
<td>11 (4.6)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>238 (100)</td>
<td>238 (100)</td>
<td>238 (100)</td>
</tr>
</tbody>
</table>

It was only one horse that did not have both its testes down at six months of age. This gives an incidence of cryptorchidism of 0.4 % when missing values were included. With horses without answers excluded, the proportion of cryptorchid horses was between 1 and 2.9 %. The majority of the answers were “not know” and “blank”. In most cases “blank” can be assumed to mean the same as “not know”. Especially at one and six month of age there were few that knew anything about their horses’ testes descent. One of the horses had had its testes down up to 1.5 year of age but then pulled them up. The horse is not included in the category of cryptorchid horses, it is instead said to have retractile testes and is included in the “yes” category.

At the study visit to the Standardbred stud, horses between three months of age and up to 17 months of age were examined by palpation. Some of them did not have both their testes down, especially the youngest ones. According to the staff the testis descent is in most cases not examined, sometimes not even before the horse is sold. Usually, only horses that are sold on auction are examined by a veterinarian before being sold.

6.2 Icelandic horses

6.2.1 Answering frequency from the questionnaire

The answering frequency for the Icelandic horses was 56.6 % (43/76). Four mails came back because the address was not correct. Additionally two answers were sent in by e-mail. Also one breeder called and said that he could not answer the questionnaire since he did not keep track of all his horses’ testes descent. The survey resulted in answers for 864 horses which gives an average of 19 horses per breeder. Six horses were excluded from the study; three of them had died young and three of them had retractile testes, leaving 858 horses.

6.2.1.1 Part 1

In Table 2 the answering frequency for part 1 of the questionnaire is showed (percentage in brackets, in total respectively with missing values excluded).
Some of the breeders did not fill in all boxes, for example they put “no” for the horse at 6 months but skipped to fill in the box for 1 month. Sometimes they also answered “not know” for 1 month but “no” for 6 months. Then it is assumed that it should be “no” also for 1 month. Also if they had filled in “yes” for 1 month, it is assumed that it is “yes” also for 6 and 12 months even if those boxes were empty. In most cases a blank answer can be assumed to be “not know” and in the Table “blank” and “not know” are presented as “missing”.

Table 2. The answering frequency for Icelandic horse breeders, corrected for “blank” and “not know” answers (percentage in brackets).

<table>
<thead>
<tr>
<th>Answer</th>
<th>1 month</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
<th>24 months+</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>72 (8/45)</td>
<td>248 (29/75)</td>
<td>598 (70/91)</td>
<td>672 (78/93)</td>
<td>703 (82/94)</td>
</tr>
<tr>
<td>NO</td>
<td>88 (10/55)</td>
<td>84 (10/25)</td>
<td>57 (7/9)</td>
<td>51 (6/7)</td>
<td>48 (6/6)</td>
</tr>
<tr>
<td>MISSING</td>
<td>698 (81)</td>
<td>526 (61)</td>
<td>203 (24)</td>
<td>135 (16)</td>
<td>107 (12)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>858 (100)</td>
<td>858 (100)</td>
<td>858 (100)</td>
<td>858 (100)</td>
<td>858 (100)</td>
</tr>
</tbody>
</table>

Of the 858 horses, almost 6% had undescended testes at 24 months of age or later. The proportion of cryptorchid horses was higher when “missing” values were excluded, especially for 1 and 6 months of age. The reason why there is less difference for the older ages is that there are less “missing” answers.

6.2.1.2 Part 2

Of those Icelandic horses that the breeders ticked “no” for at 12 months of age, they filled in in part two of the questionnaire for 42 of them. For the horses that did not have both testes down at 12 months of age it was most common that they lacked one of the testes, only four horses missed both testes. Most of the horses were castrated, most commonly at 2-4 years of age. Only five of the horses that had not had their testes down at 12 months of age got their testes down by themselves later in life (between 2 - 3.5 years of age) and could then be castrated in a standard way. The rest of them had to undergo a more advanced surgery done at a veterinary clinic. In some cases, however, the veterinarian was able to pull the inguinal testis down and then castrate the horse in a standard way. Most often the breeders did not know or remember which of the testes that was retained, but for those who knew, it was more common that the left testis was retained, in twelve cases the cryptorchidism was left sided compared to nine cases of right sided cryptorchidism. It was most common that the missing testis was in the inguinal canal (22 horses) compared to three cases of abdominal cryptorchidism.

In all cases where the testis was located in the abdomen it was the right testis that was retained. The most common remarks reported were size differences of the testes. Except for one horse that had the testis located in the abdomen enwrapped like a tumor (probably teratoma) and one horse had some problems with adherences.

6.2.2 Number of sires and dams

The horses in this study derive from totally 230 sires and 471 dams. The sires are fathers of between 1 and 36 of the horses in the study. Figure 2 shows the number of male offspring for each sire. Most of the sires had very few male offspring; 100 of them (> 40 %) had only one offspring and only 15 had more than ten offspring. For the dams the result is presented in Figure 3. The dams in this study have had between 1 and 10 male offspring and almost 58 % of them had only one offspring.
The breeders that answered the questionnaire ran studs of different size, the smallest one had only had four male foals since 1990 while the largest breeder had had 77 male foals. Figure 4 shows the distribution of number of horses for the different breeders. Most of the breeders had produced between 11-20 horses and there were few that had produced more than 40 horses.
The horses in this study were born between 1990 and 2011. In Figure 5 the number of horses born per year is shown. The majority of the horses are born during the 21st century and in this graph it can be seen that the number of horses has increased during the last 20 years, reflecting the increased popularity of Icelandic horses in Sweden.

6.2.3 Different factors and their effect on cryptorchidism

Here follows a short summary of the traits that were tested for to find out if they had any effect on the frequency of cryptorchidism in the horse. Since there were very many “missing” answers for month 1 and 6, the following results only include horses with information about crypt12, crypt24 and crypt24+.

6.2.3.1 Farm

The number of Icelandic male horses per farm during the years 1990 to 2011 is shown in Figure 6. Only horses with information on cryptorchidism at the different ages is included, so for example horses that only had information for crypt12 are not included in the numbers for crypt24 and crypt24+. In Figure 7 the proportion of cryptorchid horses per farm at different ages is presented.
6.2.3.2 Height at withers

The BLUP-values for withers height for the horses varied between -3.1 and 3.6 as showed in Figure 8. Most of the horses had a breeding value for withers height close to average and the curve is almost normally distributed. When analyzing the effect of withers height the different values were divided into different classes as presented in Figure 9. There were quite few horses that had very high or very low values.
In Figure 10 the proportion of cryptorchid horses with different BLUP-values for withers height is shown. A higher breeding value for withers height increased the proportion of cryptorchid horses.
6.2.3.3 Inbreeding coefficient

As shown in Figure 11 most of the Icelandic horses in the study had a low inbreeding value and only 7 of the horses had a value higher than 10. The value varied between 0 up to almost 16%. In Figure 12 the proportion of cryptorchid horses with different inbreeding coefficients is presented. Except for horses with an inbreeding coefficient of 2-4% there is no major difference between the different classes.
Figure 12. The proportion of cryptorchid horses in different classes of inbreeding coefficients at different ages.

For the dams in the study the inbreeding level varied between 0 and 30 percent. Most of the dams had a low inbreeding coefficient and there were only 16 horses that had a higher value than 8, as seen in Figure 13. In Figure 14 the proportion of cryptorchid horses is shown. There were seven dams that did not have any values calculated for inbreeding. Most of the horses in this study were after mares with a low inbreeding coefficient and the proportion of cryptorchidism was lowest for the mares with the highest inbreeding values.

Figure 13. The number of males with different inbreeding coefficients for the dams.
6.2.3.4 Coat Colour

Among the Icelandic horses there were 65 pinto horses, 38 grey horses and 37 horses that had the silver colour. There were also 333 horses that had white markings, either on the head or on the legs.

No effects of colour on the proportion of cryptorchidism were found. For both pinto, silver and grey the proportion of cryptorchid horses was higher for the group without the colours, but the results were not significant. For white markings the proportion cryptorchids was quite similar for horses with respectively without markings.

6.2.3.5 Foaling age

The foaling age of the dams is presented in Figure 15. As there were very few dams that had had an offspring before four years of age or later than 20 years of age, those horses are included in the class four respectively 20 years of age.

In Figure 16 the proportion of cryptorchid horses is shown for different foaling ages of the dam.
6.2.3.6 Birth year
The proportion of cryptorchid horses has increased during the last years as shown in Figure 17.

6.2.3.7 Country of origin
The country of birth for the individual horses in this study was not taken into account since all horses were born in Sweden. So only the birth country of the dams and the sires was studied. More than 50 % of the horses in this study was after sires and dams born on Iceland, about 40 % was after Swedish horses and only a few horses derives from other countries than Sweden or Iceland, for example Denmark, Norway and Germany.

The frequency of cryptorchidism was a bit higher for horses with dams not born in Sweden or Iceland, but this is probably due to very few horses in that group. Among dams born in Sweden the frequency of cryptorchidism was a bit higher compared to the group with Icelandic dams, but the difference was not significant.
For the sires the proportion of cryptorchid offspring was higher for Swedish sires and lowest for sires born in other countries than Sweden or Iceland but it was not a significant difference.

6.2.3.8 Significant factors
It was found that three factors had a significant effect on the frequency of cryptorchidism; breeding value for height at withers for crypt24 and crypt24+ (analyzed in classes), birth year at all ages (analyzed as a regression) and farm (random factor). The effect of farm was not significant for crypt24.

In the data, horses with higher breeding values for withers height more often were cryptorchids and the frequency of cryptorchidism has increased during the last 20 years. The effect of withers height was not significant for crypt12 and the p-value varied between 0.15 and 0.21 depending on the variables used in the model, but for crypt24 and crypt24+ the p-values were lower than 0.05. The p-values for birth year were lower than 0.05 for all traits analyzed.

6.2.4 Genetic parameters
The genetic and the residual variance for the different ages are shown in Table 3 (standard errors in brackets).

<table>
<thead>
<tr>
<th>Variance</th>
<th>Crypt6</th>
<th>Crypt12</th>
<th>Crypt24</th>
<th>Crypt24+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>0.022 (0.019)</td>
<td>0.009 (0.008)</td>
<td>0.019 (0.009)</td>
<td>0.005 (0.006)</td>
</tr>
<tr>
<td>Residual</td>
<td>0.071 (0.016)</td>
<td>0.059 (0.008)</td>
<td>0.047 (0.008)</td>
<td>0.053 (0.006)</td>
</tr>
</tbody>
</table>

The heritability on the visible scale was estimated to be between 0.08 and 0.28 as shown in Table 4. When converted to the underlying scale the heritability was between 0.3 and 1 for the different traits.

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Crypt6</th>
<th>Crypt12</th>
<th>Crypt24</th>
<th>Crypt24+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible scale</td>
<td>0.24 (0.19)</td>
<td>0.14 (0.12)</td>
<td>0.28 (0.13)</td>
<td>0.08 (0.10)</td>
</tr>
<tr>
<td>Underlying scale</td>
<td>0.45 (0.36)</td>
<td>0.43 (0.36)</td>
<td>1.01 (0.46)</td>
<td>0.30 (0.37)</td>
</tr>
</tbody>
</table>

7. Discussion

7.1 Answering frequency for the questionnaire
The answering frequency for the questionnaire for the Icelandic horses was almost 57 %, which is higher than expected. Engsäll (2011) found it difficult to find enough data about cryptorchidism and when this study started it was thought that it would be difficult to get as much information as needed for estimation of genetic parameters. It was thought that maybe the breeders do not know or do not want to tell if they have successful stallions that give many cryptorchid offspring. In the beginning of this study it was also discussed if the questionnaire should have been sent to horse owners instead of breeders, but most owners buy their horses when they are older than one year and then they will probably not have information about when the testes have descended.

On the other hand, the answering frequency for the Standardbred trotters was lower than expected and it was seen that most often even large Standardbred horse breeders do not know anything about their horses’ testes descent. That is probably due to that Standardbred horse breeders do not examine their horses’ testes to the same extent as Icelandic horse breeders do.
One reason why breeders of Standardbred horses have that little knowledge about the testis descent could be that it is quite common to use stallions for competition. Maybe it is less common to castrate Standardbreds compared to Icelandic horses. It could also be that the problem with cryptorchidism is not as common in the Standardbreds as in the Icelandic horse breed. Some of the Standardbred breeders said that they do not examine the testes but are willing to start doing that if asked for, which gives an indication that they just have not thought of it before and that the problem is not that big for them. Another reason for why there were fewer answers from the Standardbred breeders than the Icelandic breeders could be that they have bred many more horses, some of the breeders had bred more than 700 male horses during the last 20 years, and remember and filling in information for all those horses is difficult and take much time and effort, if they do not have it registered in journals. According to Amann & Veeramachaneni (2006) breeders of racehorses use their valuable horses for breeding even if they miss one testis. They remove the undescended testis and continue to breed on the horse. It is not known how common it is to do that in Sweden.

7.2 Frequency of cryptorchidism

The frequency of cryptorchidism among the Standardbred horses seems to be low and that agree with the result from the study by Engsåll (2011) who found that on four different animal hospitals in Sweden eight percent of the castrated Standardbreds was cryptorchid, compared to 43 % of the Icelandic horses. An explanation for the high numbers in that study is probably that horses with both testes down in the scrotum often are castrated at home, and when there are any abnormalities or Standardbred trotters older than two year or with wide inguinal canals they are castrated at an animal hospital. This is supported by the fact that only about 3 % of the Icelandic and Standardbred stallions were castrated at one of the veterinary hospitals examined. So studies on animal hospitals often give overestimated results. In this study the frequency of crypt12, crypt24 respectively crypt24+ were about 6-9 % and for the Standardbred trotter it was around 1-2 %, which is well corresponding with the numbers from the literature (reviewed by Amann & Veeramachaneni, 2007) but the numbers for the Standardbred horses are very uncertain because of the very low answering frequency.

7.3 High numbers of missing answers

The number of missing answers for 1 and 6 months were very high for both Standardbred and Icelandic horses. For the Standardbred horses the proportion of “yes” answers at 1 month was 13 % and for the Icelandic horses the proportion was about eight percent. It could be that breeders more often remember horses that have had problems and quite many of the missing answers could possibly be seen as “yes” answers. When excluding the “missing” answers more than 50 % of the Icelandic horses did not have their testes down at 1 month and for 6 months the number was 25 %. This does not match up with the information from the literature where it says that the testes should be down in the scrotum some time before birth or up to two weeks after (Bergin et al., 1970; Leipold et al., 1986; Lu, 2005). However it can be difficult to see if both testes have descended or not and some horses might pull the testes up, for example when playing with other horses or when the testes is palpated. The horse might then have retractile testes, which is not the same as cryptorchidism.

7.4 Definition of cryptorchidism

The time definition of cryptorchidism is not totally clear, according to the literature the testes should be down early after birth (Bergin et al., 1970; Leipold et al., 1986; Lu, 2005), otherwise the horse is said to be cryptorchid, but some people say that it is dependent on breed and for example Icelandic horses can be said to be normal if the testes is down before
one year of age (Axelsson, J. pers. comm., 2012). When talking to breeders and veterinarians, there are very different opinions about what is a normal time for testis descent. Some say that one month is the limit and others that one or two years are normal. There are several factors that are believed to affect the testes descent, for example different physical factors like the location of the testes in the abdomen, the abdominal pressure (Bergin et al., 1970) and also genetic and hormonal factors (Hayes, 1986). There is still a need of more studies on which factors that affect cryptorchidism.

7.5 Evaluation of methods used
The method used in this study seemed to work rather well as it is possible to get answers from many different farms without having to examine all horses by hand, but a disadvantage is that sometimes the breeder does not know or remember correctly. Breeders may also not be that experienced in examining testes, which can be quite difficult sometimes, and that can give misleading results. One disadvantage with studying a trait like cryptorchidism is that it is sex-linked and that leaves only half the number of offspring to be used in the analysis.

In the study there was no question about when the testes came down if it was later than 12 months. That was mistake, since some of the Icelandic horse breeders did not examine their horses before two years of age, when it was time for castration. Also, if a cryptorchid horse was castrated early, it is impossible to know if and when the testes would have descended otherwise. When analyzing the results two additional categories, crypt24 and crypt24+, was created since there were many breeders that only had information for those, but it would have been better to include all those categories in the questionnaire from the beginning. Normally horses are castrated between one and two years of age (Dalin, A-M, pers. comm., 2012) but in this study some of the breeders said that if one stallion does not have its testes down at that age they wait until the testes has descended and then castrate the horse in a standard way.

7.6 Aetiology and inheritance pattern
Cryptorchidism is probably affected by many different genes and maybe it is not the same genes that control if the testes are descended late or if they are located in the inguinal canal or in abdomen and does not descend at all. One Icelandic horse breeder in the study said that he just wait until the testes come down and if the stallion has very good qualities he is still used in breeding. If the defect is heritable, which it seems to be, it could mean that the offspring will have their testes down later than normal and this may give later and later testis descendent.

The fact that crossbred horses seem to have higher risk for being cryptorchid (Hayes, 1986) is interesting. If the defect is recessive it would be expected that crossbred horses have lower risk since they are less inbred. Maybe crossbred horses have larger testes due to heterosis effects and that it affects the testes passage through the inguinal canal or maybe the defect is dominant and expressed more in crossbred horses. However, the fact that mating of two so-called carriers was significantly different from mating two non-carriers, and that mating one carrier with a non-carrier was not significantly different (Gubbels et al., 2009), indicates that the inheritance pattern might be autosomal recessive. This is also supported by the fact that the incidence of cryptorchidism was much higher in the groups with litters from two carriers compared to the whole population.

7.7 Factors affecting cryptorchidism
When analyzing the effect of birth year it can be seen that the frequency of cryptorchidism in the Icelandic breed has increased during the last years. One reason for the significant effect of
birth year in this study might be that the breeders in the study remember more about the horses that are born later. It could also be that there are some popular stallions that have been used that might give increased frequencies of cryptorchidism. Another reason could that people are more aware of the problem today and examine the stallion more carefully. But it could of course also be that the problem has increased.

In this study it was found that breeding value for height at withers had a significant effect on cryptorchidism for crypt24 and crypt24+; a higher breeding value increased the frequency. The effect was not significant for crypt12 but this could be because there were fewer horses that had information for 12 months compared to 24 and 24 months+. The effect of height at withers could be due to chance: that there are some stallions with a high breeding value for withers height and also a higher risk for inheriting the defect. It would have been interesting to know the exact withers height for all horses since there are many horses that have not been measured. The results from this study is the opposite from what have been found in earlier studies, here a higher breeding value for withers height increased the frequency of cryptorchidism while other studies have found that smaller animals have higher risk for getting the defect, for example ponies and small dogs (Cox et al., 1979; Hayes et al., 1985; Hayes, 1986). Small breeds have a faster relative growth rate than larger breeds (Rundgren, M. pers. comm., 2012) and maybe large horses also are later in their development, and get their testes down at older ages. However it would be interesting to test it on a larger data material, preferably on different breeds, to see if the effect is still significant.

The colour of the horses did not seem to have an effect on the frequency of cryptorchidism but this might be due to the small animal material. It might be interesting to examine it further, especially the colour pinto and white markings, since it has been found on mice that it might have an effect (ESPCR, 2012). Even if it did not give any significant results in this study it might have been different if the data material had been bigger. The p-value for pinto was lower than the value for grey and silver horses. In this study it was not examined if the colour of the mares had any effect on cryptorchidism but this would be interesting to do in a future study. For example one of the mares in the study that was roan had had several cryptorchid male offspring.

**7.8 Heritability estimations**

The heritability was estimated to between 0.3 and 1 on the underlying scale, with high standard errors, because of the small data material. The extremely high heritability for crypt24 is not reasonable, but why it was so high is hard to tell, there was no large difference from the category crypt24+, both the number of horses and the frequency of cryptorchidism are quite similar. Maybe some specific dams or sires that have offspring with observations for crypt24 but not for crypt24+ might affect the result, but there were no obvious signs of that in the data. The other heritabilities found in this study (0.3 respectively 0.5) are comparable with earlier studies on pigs and dogs. Most of them have found moderately high heritabilities (Mikami & Fredeen, 1979; Nielen et al., 2001; Dolf et al., 2010; Mattson, 2011).

The heritability estimation in this study is interesting since it is the first estimation for horses. It was not significant but it is an indication that the defect probably is heritable. It has been suggested that a maternal effect could be important for cryptorchidism and some of the mares in this study have had several offspring with late testis descent. Some of the maternal effects might be included in the effects of farm, since most of the dams in the study have been kept on only one farm. The significant effect of farm maybe compensates for the fact that the
examinations of the testes are made by different people. The fact that some mares got several cryptorchid offspring might be due to different genes, but there are also a lot of factors in the environment that might influence the intra-uterine environment. It is more difficult to estimate the effect of the environment for the dams compared to the stallions. This is because offspring from the same mares most often are born in the same place, fed the same feed, kept on the same pastures and so on, so there are a lot of factors that might affect the testes, both during pregnancy and also after birth.

From the beginning it was not planned to estimate heritability for cryptorchidism at 6 months since there were so few horses, but just to see if the value differed from the other the heritability was estimated also for 6 months of age.

7.9 Results comparable with literature

Of the horses that did not have both testes down at 12 months of age, and where the breeder answered part two of the questionnaire, it was most common that the horses were unilateral which is also most common according to the literature (Coryn et al., 1981). Earlier studies have found that in pony breeds inguinal cryptorchidism is more common on the left side and the right testis is more often located in the abdomen (Cox et al., 1979; Coryn et al., 1981). This was also found in this study, in all cases were the testis was located in the abdomen it was the right one that was missing. Among the horses in the study it was most common that the left testis was missing and this can be due to the size difference, since the left testis is often larger than the right (Leipold et al., 1986) and normally descend slower into the scrotum than the right testis (reviewed by Mueller & Parks, 1999).

It has been shown in earlier studies that cryptorchid testes usually are smaller than normal (Leipold et al., 1986; Bøgh et al., 2001) with a different texture (Leipold et al., 1986) and that was also found in this study. The most common defects that were reported in this study were size differences of the testes and only a few of the horses had any other problems.

7.10 Information from US Trotting Association

In the study it was difficult to get information about the testis descent in the Swedish Standardbred breed. It was examined if it was possible to get information about the American Standardbred breed since ridglings is one of the gender options at the foal registration. Information about ridglings registered in USA was given by the USTA but since many of the horses registered as geldings might have been cryptorchid earlier in life it was not possible to use the information in any analyzes. If the original gender would have been easier to find the material could have been used in this study. It is good that ridgling is one of the gender options and maybe it could be registered also at the Swedish Trotting Association in the future. But then it is important that the original gender is saved even if the horse is castrated later in life. However, one question could be whether the breeder or the foal owner really has the interest and knowledge to examine the horses’ testes in a careful way or if they just register it as a stallion.

7.11 Future studies

It might be interesting conduct future studies on other breeds, for example the Shetland pony or Welsh to find out if the results differ a lot from the result in this study. Shetland ponies also have a lot of different colours and it might be possible to see if there are any significant effects of different colours on the frequency of cryptorchidism. With a larger data set more factors may be found to have an effect and further on it would be of great interest to find out which genes are causing the defect.
Since there were very few answers for Standardbred trotters these answers could not be used for further analyses, but it may be possible to use large Standardbred horse farms for recording information for future studies about cryptorchidism, since they often have many horses and good registration possibilities. Many larger Standardbred studs have their own veterinarian that visit the stable several times a week. The best thing for such a study would be to let for example a veterinarian examine all horses’ testes, for example once a month during a two year period. That would give much more reliable results since all horses would be examined by the same person and it would be possible to get a more exact date for when the testes have descended.

7.12 Recommendations
Since cryptorchidism probably is heritable the recommendations will be to not use cryptorchid stallions or stallions that had had their testes down late, in breeding, even if they have other good qualities. This is especially important in breeds that seem to be more affected by the defect, like the Icelandic horse.

8. Conclusions
Three factors had a significant effect on the probability of cryptorchidism; breeding value for withers height, farm and birth year. The heritability for equine cryptorchidism was estimated to 0.1-0.3 on the visible scale and 0.3-0.5 on the underlying scale, using different definitions with different ages. The estimated heritability at the underlying scale for cryptorchidism at 24 months was unreasonably high (1.0) and the reason why is still unknown. The estimated heritabilities were not significant and more studies are needed on larger data material. There were indications that cryptorchidism is more common in the Icelandic breed compared to Standardbred trotters.

9. Acknowledgement
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Tillsammans kan alla som medverkar bidra till bättre kunskap om den genetiska bakgrunden för kryptorkism.

Vänligen mejla den ifyllda enkäten till Kim Jäderkvist, kija0001@stud.slu.se alternativt skriv ut enkäten och skicka med post till:

Gabriella Lindgren  
SLU, Institutionen för husdjursgenetik  
BMC Box 597  
751 24 Uppsala

Hela forskargruppen tackar i förskott för Er insats!

Vänliga hälsningar,
Kim Jäderkvist, Susanne Eriksson, Gabriella Lindgren och Jeanette Axelsson
Genetisk studie av kryptorkism hos häst

**Bakgrund:** Kryptorkism är en medfödd defekt hos hingstar och dessa benämns ofta klapphingstar. Defekten innebär att en eller båda testiklarna ligger kvar i bukhålan eller i inguinalkanalen (mellan bukhålan och pungen) efter födseln i stället för i pungen. De flesta avelsorganisationerna tillåter inte avel med klapphingstar. Kryptorkism leder även till ökade kostnader för kastrering samt en ökad risk för att den kryptorkida testikeln tumöromvandlas om den inte tas bort. Eftersom defekten troligen är relativt vanlig vill vi genomföra en studie om den genetiska bakgrunden för kryptorkism i form av ett examensarbete vid Sveriges Lantbruksuniversitet (SLU).

**Mål:** Syftet med den här enkäten är att samla information som grund för en genetisk studie av kryptorkism. Målet är att senare även att kunna identifiera de gener som orsakar defekten.

**Vi behöver din hjälp:** Vi behöver därför information om när testiklar vandrat ner i pungen hos många hingstar/valacker. Genom att besvara denna enkät, som vi skickat ut till större uppfödare av varmblodstravare och islandshästar i Sverige, kan Du bidra till att forskningen kring defekten går framåt. Det är av stor vikt för oss att få in så många svar som möjligt. Projektet är finansierat av forskningsrådet FORMAS, Stiftelsen Hästforskning och Carl Tryggers Stiftelse.

**Vi behandlar svaren konfidentiellt:** Inga enskilda hingstar/valacker eller uppfödare kommer att omnämnas i arbetet utan det är rasen, d.v.s. populationen, som är av intresse. Alla uppgifter som samlas in kommer att behandlas under sekretess och endast användas i forskningssyfte.
Så här fyller du i enkäten

Svarar du nej på fråga 2 och/eller 3 (varmblod) respektive fråga 3 (islandshäst) för någon av hästarna, gå vidare till Enkät 2 och svara på resterande frågor för den hästen.

I enkäten definierar vi kryptorkism som att den ena eller båda testiklarna inte vandrat ner och finns på plats i pungen då hingsten är 6 månader gammal för varmblod och 12 månader gammal för islandshäst, oavsett om det senare rättar till sig.

Skicka gärna med en kopia på rapport från veterinär om du har det, men detta är naturligtvis inte nödvändigt.

Kontakta oss: Har du frågor om projektet eller undrar hur över du ska fylla i enkäten är Ni välkomna att kontakta:

Kim Jäderkvist, Agronomstudent vid SLU  
Telefon: 073-83 11 812  
E-post: kija0001@stud.slu.se

Gabriella Lindgren, Docent, SLU  
Telefon: 018-471 4971  
E-post: gabriella.lindgren@slu.se

Susanne Eriksson, AgrD, SLU  
Telefon: 018-67 20 07  
E-post: susanne.eriksson@slu.se

Jeanette Axelsson, Fil.Dr, SLU  
Telefon: 072-325 14 34  
E-post: Jeanette.Axelsson@slu.se

Hela forskargruppen tackar i förskott för Er insats!
**Enkät 1  Svara för både friska och drabbade hästar.**

**Ägar-/Uppfödaruppgifter**  OBS! ange minst ett telefonnummer

- Namn:
- Adress:
- Tel. hem:
- Tel. arb:
- Tel. mobil:
- E-post:
- Datum då enkäten fylls i:

**Stuteri/gårdsnamn/uppfödare:**

Hur länge har du hittills fött upp hästar?

<table>
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<tr>
<th>Hästnamn</th>
<th>ID/reg nr häst</th>
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**Hade hästen båda sina testiklar i pungen vid:**

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<th>1. 1 mån ålder</th>
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13. Appendix 3

Questionnaire part 2

V.G. Texta tydligt! Tack för din medverkan!

______________________________

Hästens namn:
Hästens ras:
Hästens ID-nummer/Reg.nummer:
Ditt namn och telefonnummer:

1. Gäller kryptorkismen ena eller båda testiklarna?
   En □    Båda □

2. Om endast en testikel är eller har varit drabbad, gäller det höger
   eller vänster?
   H □    V □    Vet ej □

3. Har/hade hästen någon av dessa avvikelser?
   A: Storleksavvikelse på testikel/testiklar □
   B: Vriden testikel/vridna testiklar □
   C: Mjuk testikel/mjuka testiklar □
   D: Annan avvikelse □ Vad?______________________________

4. Är hästen kastrerad
   Ja □    Nej □    Vet ej □
   Om Ja, vid vilken ålder kastrerades hästen?__________________

5. Om hästen är kastrerad, vid vilken klinik utfördes kastrationen,
   alternativt utfördes kastrationen i hemmamiljön?

6. Om hästen är kastrerad, var återfanns vänster testikel?
   Buk □    Inguinalkanal □    Pungen □    Vet ej □

7. Om hästen är kastrerad, var återfanns höger testikel?
   Buk □    Inguinalkanal □    Pungen □    Vet ej □