Breeding Soundness Evaluation of Young Beef Bulls

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Till Astrid och Åsa

Tjur är en individ av hankön av nötkreatur, älg med flera hovdjur.
Om tjuren är kastrerad blir den en oxe.
Kända tjurar är till exempel: Tjuren Ferdinand

Wikipedia

Abstract

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The overall aims were to learn more about factors affecting fertility in Swedish beef sires in general and in yearling beef bulls in particular, focusing mainly on sexual maturity and hind limb health finding useful tools in order to be able to routinely perform a complete bull breeding soundness evaluations in Swedish beef bulls.

Semen was collected from yearling beef bulls, postmortem, and the sperm morphology was evaluated. Using a strict definition, based on sperm morphology, we could demonstrate that less than half the number of the bulls was considered sexually mature at 12 months of age.

Samples collected by transrectal massage were compared with the two most commonly used collection methods; artificial vagina and electroejaculation, and with samples collected by cauda epididymal dissection postmortem. Sperm morphology in semen collected by transrectal massage was as representative as in samples collected by artificial vagina and electroejaculation and to some extent also in those collected postmortem. However, reduction in volume, concentration and motility would need to be considered, especially if semen is collected outdoors under suboptimal conditions. In conclusion, the transrectal massage technique is a very useful semen collection method in the field when no other collection method is possible, thus enabling a complete bull breeding soundness evaluation.

Femorotibial, femoropatellar (stifle), tarsocrural, talocalcaneus, and proximal intertarsal joints from young beef sires with impaired fertility, but no signs of lameness, were examined postmortem regarding the presence of joint lesions. A majority of the bulls had severe, bilateral lesions referred to as osteoarthritis secondary to osteochondrosis dissecans. We think that the osteoarthritis present in the hind limbs must have contributed to the infertility, rendering the bulls difficulties in mounting. The fact that most of the lesions were bilateral, could explain the absence of clinical lameness. The results of this study indicate that the poor fertility reported in these beef bulls can be a result of joint problems, secondary to osteochondrosis. Thus, joint lesions should always be taken into consideration as a contributory cause of reproductive failure in beef sires with or without symptoms of lameness.

Keywords: Bos Taurus, sexual maturity, sperm morphology, semen collection, transrectal massage, joint, osteochondrosis, osteoarthritis, artificial vagina, electroejaculation

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Appendix

Papers I-V

The thesis is based on the following papers:

I: Persson, Y. & Söderquist, L. (2005). The proportion of beef bulls in Sweden with mature spermiograms at 11 to 13 months of age. *Reproduction in Domestic Animals 40,* 131-135.

II: Persson, Y., McGowan, M. & Söderquist, L. (2006) Comparison between the sperm morphology in semen samples obtained from yearling beef bulls by transrectal massage of the ampullae and cauda epididymal dissection. *Reproduction in Domestic Animals 41,* 233-237

III: Palmer, CW., Brito, LFC., Arteaga, AA., Söderquist, L., Persson, Y. & Barth, AD. (2005) Comparison of electroejaculation and transrectal massage as methods of semen collection in range and yearling feedlot bulls. *Animal Reproduction Science 87,* 25-31.

IV: Persson, Y., Strid, G., Håård, M. & Söderquist, L. (2007). Comparison between the sperm morphology in semen samples obtained from yearling beef bulls by transrectal massage and artificial vagina. *The Veterinary Record* (accepted)

V: Persson, Y., Söderquist, L. & Ekman, S. Joint disorder; a contributory cause to reproductive failure in beef bulls? (Manuscript)

Paper I-IV are reproduced with kind permission from the journals concerned.

Abbreviations

AI	artificial insemination
AV	artificial vagina
BBSE	bull breeding soundness evaluation
CED	cauda epididymal dissection
EEJ	electroejaculation
OA	osteoarthritis
OC	osteochondrosis
OCD	osteochondrosis dissecans
SC	scrotal circumference
TM	transrectal massage

Introduction

Most Swedish beef cows and heifers are bred naturally, which makes selection of herd sires a critical decision affecting reproductive performance and profitability. It is essential that the bull is reproductively sound and produces semen of good quality. Bulls with poor fertility capacity, whether due to inability to serve or poor semen quality, cause considerable economic loss to the farmer. The fertility of an individual bull has a far greater impact on herd performance than the fertility of an individual cow (McGowan, 2004). It is, therefore, important to examine the potential fertility of beef bulls prior to sale or use. The reproductive potential of Swedish beef bulls used for natural service is at present, however, poorly documented.

Swedish beef cattle breeding

Twenty years ago, the beef cow population in Sweden was about one tenth (70 000) of the total cow population. Today beef production is an increasing agricultural sector in Sweden and about one third (177 500, 2006), of all Swedish cows are beef cows (www.sjv.se, 2007). A greater interest in beef cattle production is partly due to a declining profitability in dairy production with a subsequent increased demand for calves and beef meat and partly due to subsidies from the European Community that promote cattle pasture (Strand, personal communication 2007).

In Sweden, more than 1000 non-tested beef bulls are sold yearly, mediated by Swedish Meats or by other private treaty (Lindell, personal communication 2003). In addition, a group of about 80 top-ranked performance-tested yearling bulls are annually sold after six months of performance testing at the testing station (Gismestad) in central Sweden. The bulls are tested for post-weaning body weight gain and a limited bull breeding soundness evaluation (BBSE) is performed. Charolais, Hereford, Simmental, Aberdeen Angus, Limousine and Blonde d´Aquitaine are represented at the station. In each breed, one or two of the best bulls are annually selected for semen collection and used for artificial insemination (AI).

Yearling beef bulls and sexual maturity

There are potential benefits of using yearling beef bulls (Bertram *et al.,* 2000), mainly by increasing the profit potential, in reducing bull cost per pregnancy (Kasari *et al.,* 1996). However there are also risks involved since the sexual maturity age varies considerably. Sexual maturity of Swedish beef bulls have only been evaluated in a few studies and they indicate that Swedish yearling beef bulls have immature spermiograms (Einarsson, 1989; Söderquist, 2000) at the time they are offered for breeding purposes.

Sexual maturity is defined in various ways in the literature. One definition is that onset of puberty is “the first time semen with 50 x 10^6 sperm per ml and 10%

progressive motility can be collected" (Palasz *et al.,* 1994). A general definition is that puberty is reached when sexual organs have become fully developed, sexual instincts are prominent, and reproduction is possible (Abdel-Raouf, 1960). Another definition states that puberty is reached when the male has a pronounced libido, a mating capability, fully developed testes and a normal semen picture (Einarsson, personal communication 1996). Yet another definition, first used on boars by Bane *et al.* (1965) is that a spermiogram with <15% proximal droplets and <15% abnormal heads is considered mature. This definition has also been used for Swedish yearling beef bulls and has according to the experience in our semen reference laboratory, proved indicative of the ability for normal reproduction in beef bulls (Bane, personal communication, 1982).

Bull breeding soundness evaluation

Selection of satisfactory yearling beef sires should preferably be made on the basis of a complete bull breeding soundness evaluation (BBSE), including microscopic examination of a semen sample.

A complete routine BBSE includes the following steps (McGowan, 2004):

1. Recording the husbandry and history of the bull.
Clinical illness, lameness and significant body condition changes in the past 2 to 4 months should be noted. The levels of concentrate feeding in the past 4 months and details of the bull's previous reproductive performance should be recorded.

2. General physical examination including assessment of gait.
The bull should be adequately developed for its age and breed and be in good general health condition; free from conformational abnormalities likely to affect reproductive function, e.g. excessively straight legs. The posture and gait should be observed with the bull standing, walking and trotting freely in a yard, and particular note should be taken to the legs including the hooves.

3. Collection and evaluation of semen (see in detail below p 13-14).

4. Examination of the external and internal genitalia (including measurements of scrotal circumference).
After a visual observation, the prepuce, penis, scrotum, testicles, epidydymes and spermatic cord should be carefully palpated. Measurement of the scrotal circumference (SC) is an accurate predictor of both testicular weight and sperm output, and for yearling bulls SC should be at least 30 cm. The internal genitalia are examined by rectal palpation.

5. Assessment of mating ability and libido.
A serving and mating ability test requires observation of the bull repeatedly attempt to mount and serve restrained females. This procedure is not in use in Sweden due to ethical matters. Instead the farmers are instructed to carefully observe the bull during the first 3 weeks of mating.

Today no complete BBSE protocol is routinely used in Sweden. Measurement of the SC and a clinical examination of the genital organs are performed routinely at the performance testing station, but still not routinely by practitioners in the field. Some bulls with normal SC and normal testicular tone may still have unsatisfactory semen pictures, emphasizing the importance of including the microscopic examination, and especially the morphological examination of semen (Holroyd *et al.,* 2002), as part of the BBSE (McGowan *et al.,* 2002). However, no satisfactory methods are, at present, available that facilitate collection and evaluation of semen of potential beef sires in Sweden.

Semen collection methods

Artificial vagina

Collection of semen by using an artificial vagina (AV) is routinely used worldwide and considered the optimal way to get a normal and representative semen sample (Salisbury, 1978, Noakes *et al.,* 2001). Thus, using an AV is by far the most common method to collect semen in dairy bulls, as well as in beef bulls used for AI. However, BBSE of beef bulls are often performed under field conditions on untrained bulls, which make semen collection with AV very difficult.

Electroejaculation

Electroejaculation (EEJ) is, when performed by a skilled veterinarian, a procedure most likely to result in a semen sample suitable for examination in more than 95% of the bulls (McGowan, 2004). In many countries electroejaculation is routinely used when conducting BBSE since it is considered to be a quick, safe and reliable procedure. However, welfare considerations, due to stress or pain of EEJ, are becoming more important in many countries that use EEJ (Galloway, 1998; Mosure *et al.,* 1998; Falk *et al.,* 2001) and it is prohibited on un-anaesthetized animals in Sweden. Hence, alternative methods of semen collection are needed.

Cauda epididymal dissection

Until now, the most common method to accomplish a sperm morphology evaluation in Swedish beef bulls suspected of impaired fertility has been on semen samples collected from the cauda epididymis postmortem since EEJ is prohibited and AV is considered too difficult under field conditions. Clearly a method that only can be used postmortem has disadvantages. For example; this method does not include parameters such as volume, sperm motility and concentration. However, spermatozoa in semen samples taken from the cauda epididymidis have been reported to be as fertile as those in the ejaculate (Igboeli & Foote, 1968) and sperm morphology evaluated in samples from cauda epididymidis is considered to represent testicular, as well as epididymal function (Einarsson *et al.,* 1979; Barth & Oko, 1989). Hence, semen samples collected by cauda epididymal dissection (CED) should be regarded as representative samples from a sperm morphological point of view (Rao, 1971).

Transrectal massage

Transrectal massage (TM) directed specifically towards the ampullary region of the ductus deferens has been shown very effective for producing semen emission and semen samples (Palmer *et al.,* 2004). The method was first described by Case (1925) and later by Miller & Evans (1934). Parsonson *et al.* (1971) reported a similar method for collection of seminal fluids for microbiologic examination and suggested that the method could also be used for semen evaluation in bulls. Semen collection by TM has been described in cattle (Case, 1925; Miller & Evans, 1934; Goodwin, 1970; Salisbury *et al.,* 1978; Wolfe, 2001; Palmer *et al.,* 2004), elephants (Schmitt & Hildebrandt, 1998) and man (Fahmy *et al.,* 1999; Okada *et al.,* 2001). Most bulls do not object vigorously to this collection method (Wolfe, 2001) and prior training of the bulls is unnecessary. Hence, semen collection by TM might be a suitable method to use under field conditions by veterinary practitioners where other semen collection methods are less suitable.

Transrectal massage has previously not been fully evaluated for use in yearling beef (12 months old) bulls. Furthermore, there are no studies evaluating how well semen sample collected by TM correspond to semen samples, obtained by other methods such as EEJ and AV.

Evaluation of semen quality (see in detail in the methodological section)

Immediately after the semen has been collected, the individual, progressive sperm motility must be assessed. A fair semen sample, collected in the field by EEJ, needs to have 40-59% individual motile spermatozoa (60-69% for a good sample and 80-100% for a very good sample) (Barth, 2000). Concentration of the semen sample is evaluated by using a photometer or a haemocytometer. A fair semen sample collected in the field by EEJ should have a concentration exceeding 250 million sperm per mL (a good sample at least 400 million sperm per mL and a very good sample at least 750 million sperm per mL) (Barth, 2000). In a BBSE in the field, a good indirect determination of the sperm concentration is measurement of the SC since it is a predictor of the sperm output. Sperm morphology is evaluated either directly in the field or at the laboratory, and different countries have different morphological classification systems. One of the most accepted definitions of a normal spermiogram is that at least 70% of the spermatozoa should be normal (Barth, 2000; Fitzpatrick *et al.,* 2002). Unfortunately, the incidence of normal spermatozoa is not defined in our classification system at our reference laboratory. This is because routinely the sperm abnormalities are recorded with two overlapping methods, dry smears stained with carbol-fuchsine and wet-mount preparations of formol saline (4-5% aqueous solution of buffered formaldehyde) fixed spermatozoa. This means that the same spermatozoa can be recorded several times for different abnormalities and as a consequence, the calculation of the percentage of normal spermatozoa becomes uncertain (Selin-Wretling, personal communication, 2006). Instead, a recommendation for semen samples from beef bulls routinely used in our reference lab is that none of the sperm defects registered must exceed 15% for a semen sample to be classified as a normal sample and approved at our laboratory (Bane, personal communication, 1982).

Skeletal soundness

Lameness, whether due to musculoskeletal abnormalities or claw lesions, negatively affects the bull's ability to mount and hence the reproductive potential of the bull. The lower fertility within the herd lowers the profit considerably for the farmer (Bartels, 1975). Since most beef sires are used for natural service, the limbs, and in particular the hind limbs are important. Earlier reports on lameness, as a cause of infertility in bulls, have described vertebral changes in dairy bulls (Bane & Hansen, 1962), degenerative joint disease in beef bulls (Bellenger, 1971) and in dairy bulls (McEntee, 1958). In beef bulls, the most common cause of lameness is probably osteochondrosis (OC) with subsequent osteoarthritis (OA), but reports on OC in cattle are few, mostly done postmortem and have mainly been described in studies evaluating diet (Trostle *et al.,* 1997). In a group of healthy, performance-tested yearling beef bulls, representing the most genetically valuable beef animals in Sweden, 97.8% had joint lesions at slaughter, compatible with subclinical OC (Dutra *et al.,* 1999). To our knowledge, there are no studies on how OC and OA contribute to impaired fertility in beef bulls.

Osteochondrosis

Osteochondrosis (OC) is considered a multifactorial condition that affects the immature skeleton of many species, including cattle (Tryon & Farrow, 1999). It can manifest both in the metaphyseal growth plate and in the epiphyseal articular cartilage complex (Bailey, 1985). The aetiology is not fully understood, but it is suggested that focal failure of blood supply in the growth cartilage causes local ischemia, which in turn leads to focal necrosis of the cartilage; this is defined as OC latens (Ytrehus *et al.,* 2004). This focal change can develop into cartilage retention in the subchondral bone and is defined as OC manifesta (for review, see Ytrehus *et al.*, 2007). The disorder is frequently seen in pigs (Grondalen, 1974; Reiland, 1978), horses (Rejnö & Strömberg, 1978), and dogs (Olsson, 1987), but has also been described in man (Flynn *et al.,* 2004), poultry (Poulous *et al.,* 1978), rat (Kato & Onodera, 1987), cat (Ralphs, 2005) and cattle (Trostle *et al.,* 1998). Heredity, gender, growth rate, body weight, trauma, nutritional imbalance and anatomical conformation have been proposed as aetiological factors (for review see Ekman & Carlson, 1998; Ytrehus et al., 2007). The joint shape, growth rate and body weight have been suggested as factors influencing the local conditions of the tissue (Ytrehus *et al.,* 2004). Osteochondrosis is more often seen in males than in females (Jubb *et al.,* 1993). Hill *et al.* (1998) reported that inheritance and gender were the most possible factors that contributed to OC in a group of Brahman bulls kept on extensive grazing. Earlier studies have described the correlation between intense feeding regimens, rapid growth rate and OC lesions in cattle (Reiland *et al.,* 1978; Davies *et al.,* 1996). Sub-optimal mineral and vitamin inclusion in the diet have also been proposed as predisposing factors (Davies *et al.,* 1996). Hard flooring (White *et al.,* 1984; Jubb *et al.,* 1993) and lack of exercise (Reiland *et al.,* 1978) have also been described as promoting the development of OC in cattle.

Osteochondrosis may be present without causing any clinical symptoms or only a mild progressive lameness (Bailey, 1985), partly because bilateral lesions are common in bulls (Trostle *et al.,* 1998) and, hence, lameness can be difficult to observe under field conditions. The focal necrosis (latens) of growth cartilage with impaired ossification (manifesta) is sometimes followed by osteochondrosis dissecans (OCD) (Olsson, 1978). Osteochondrosis dissecans is a term that describes an OC disorder that includes splitting or separation of pieces of cartilage into a joint (Baxter *et al.,* 1991). The development of OCD can be related to factors that influence the dynamics of the joint, such as conformation and body weight. Transportation or mounting may exacerbate the cartilaginous flap, producing a sudden onset of lameness as the main clinical symptom (Bailey, 1985). OCD causes a synovitis and secondary OA (Olsson, 1978). The stifle is the most common joint affected in the bull (Trostle *et al.,* 1998). Radiological examination is possible, but according to our experience, difficult to perform in the beef bull, mainly due to the thickness of the muscles surrounding the stifle. Moreover, joint effusion, a common clinical sign in horses (van Weeren, 2006, review) with OC of the stifle joint, is difficult to evaluate in the beef bull. It can be therefore problematic to determine if poor fertility within a herd is caused by hind limb disorder of the bull

Aims

The overall aims of this thesis were to learn more about factors affecting fertility of Swedish beef sires in general and of yearling beef bulls in particular, focusing mainly on sexual maturity and hind limb health, and to find useful tools to be able to routinely perform a complete bull breeding soundness evaluation in beef bulls in Sweden.

Specific hypothesises of the thesis were:

- Swedish yearling beef bulls are not fully sexually mature at the age of 12 months, when they are offered for breeding purpose

- Semen collection by the transrectal massage method in yearling bulls would provide a representative semen sample for a correct evaluation of the spermiogram, similar to collection with an artificial vagina or by electroejaculation.

- Osteochondrosis and osteoarthritis contribute to poor fertility in beef bulls.

Methodological considerations

Materials and methods used in the present studies are presented in details in the **Papers (I-IV)** listed in the appendix above. A generalised description of materials and methods is presented here.

Animals (Paper I-V)

General information

All bulls in this thesis were purebred beef sires intended, or used, for breeding purposes. Bulls either originated from (**IV**) or were housed at (**I-II**) the only performance testing station (Gismestad) for beef bulls in Sweden (see in detail below). Yearling, purebred beef bulls with clinically normal reproductive organs and a scrotal circumference (SC) above 30 cm (Chenoweth, 1993) were included in these three studies (**I, II, IV**). In **Paper I**, 142 yearling beef bulls (median age of 12 months) were culled after six months of performance testing (due to growth rates below the stated thresholds), and semen samples were collected by cauda epididymal dissection (CED) postmortem. In **Paper II**, semen samples were collected by transrectal massage (TM) from 52 yearling beef bulls (median age of 12 months) and from 23 of these bulls that were culled also by CED postmortem. In **Paper IV**, semen samples were obtained by TM and artificial vagina (AV) from 14 yearling beef bulls (median age of 15 months). The 34 bulls in **Paper V** were slaughtered during or after the breeding season (mean age of 2.5 years) due to poor fertility results. Lameness had not been observed. Semen samples were obtained from 26 of these bulls by CED postmortem. As a control, 11 beef bulls (mean age of 4.5 years) with good fertility result were included. In **Paper III**, 137 range beef bulls (aged 1-9 years) were assigned to semen collection by electroejaculation (EEJ) or TM on an alternate basis.

The performance testing station ***(Paper I, II and IV)***

Gismestad performance testing station, outside Linköping, central Sweden, is the only testing station for beef bulls in Sweden. Between 160-180 beef bulls are tested each year for post weaning body weight gain. Weaned bull calves from top ranked pedigree herds enter the station at about six months of age. On arrival, they are divided into groups of 10 to 20 animals, based on breed and body weight. Each group is kept in a semi-outdoor pen with concrete floor and straw as bedding. They are fed a complete-ration feed *ad libitum*, with approximately 60% of the energy provided by silage and 40% by concentrate. The bulls are weighed every second week throughout the testing period (September-March) and at the end of the period, an individual growth index is calculated. Besides growth rate, general health, conformation, temperament, scrotal circumference (SC) and gait are also evaluated. However, only growth rate is included in the bulls breeding index. Bulls with either growth index below the stated threshold for each breed, or a small SC or testicular abnormalities, are either slaughtered or returned to their owners. Bulls with normal reproductive tracts (normal appearance and consistency of testicles, epididymes, spermatic cords, penis and prepuce and an SC above a given threshold) and fast growth rates are sold at the livestock auction at an age of between 11 to 14 months.

Clinical examination ***(Paper I, II and IV)***

The bulls were examined at Gismestad performance testing station by the station veterinarian at the beginning and end of the testing period. A general clinical examination was performed, together with a special clinical examination of the external genital tract. The second examination included inspection and palpation of the testes and epididymides, inspection of the prepuce and measurement of the SC. During measurement, the bulls were restrained in a chute to minimize forward, backward and lateral movements. Only bulls with normal genital organs and an SC above a given threshold for each breed and age were included in the study.

Semen collection (Paper I-V)

*Semen collection environment (**Paper II-IV**)*

Semen was collected from bulls at Gismestad performance testing station during two days in February in a semi-outdoor pen (**II**). The outside weather conditions were cold (mean temperature -15ºC), windy and snowy the first day of collection, followed by warmer conditions (mean temperature -5ºC) the second day. In **Paper IV**, semen was collected from beef bulls, indoors in room temperature at an AI centre in Falkenberg, southwest Sweden. In **Paper III**, semen was collected outdoors from bulls in three community pastures in western Canada, during early spring with an outdoor temperature close to 15ºC in the early mornings, rising to a high of 25ºC at most.

*Cauda epididymal dissection (**Paper I, II and V**)*

The bulls **(I, II)** were slaughtered as yearlings (median age 12 months) before the breeding season due to inferior growth rates, whereas the bulls in **Paper V** were slaughtered after being used during at least one breeding season. Testes and epididymides were removed at slaughter, immediately put in a container with crushed ice, transported in a refrigerated truck to the lab for arrival the next day, where they were examined macroscopically and weighed. An incision (approx. 1.5 cm long and 0.5 cm deep) was made with a scalpel blade in the middle, distal part of the cauda (Gustafsson & Crabo, 1971), and aliquots of the caudae epididymal contents were collected with a plastic pipette. Dry smears were prepared and aliquots were also fixed in formol saline (4-5% aqueous solution of buffered formaldehyde) for sperm morphology evaluation.

Collection of semen samples by transrectal massage of the ampullae ***(Paper II-IV)***

The bulls were restrained during collection of semen by TM of the ampullae. In brief, the operator inserts a hand into the rectum and then alternately massages the ampullae firmly, and then rhythmically strokes the urethralis muscles (McGowan *et al.,* 1995; Palmer *et al.,* 2004). An assistant collected the semen as it was emitted from the preputial orifice into a plastic bag suspended in a thermos containing warm water at the bottom, resulting in a temperature of 35-38ºC in the plastic bag. The massage time for each bull was recorded from start of massaging until an opaque to milky sample of fluid was collected (**Paper II and IV**) or stopped after 4 minutes (**Paper III**). Immediately after collection, the progressive motility was evaluated under a light microscope equipped with a heated stage (37ºC) at magnification x 200 (**Paper III**; x 400). The volume of each sample was measured in a graduated test tube and dry smears were prepared. Aliquots were also taken and fixed in formol saline (4-5% aqueous solution of buffered formaldehyde) for sperm morphology examination (**Paper II**). The remaining part of the semen sample was used for assessment of the sperm concentration.

*Collection of semen samples by electroejaculation (**Paper III**)*

Electroejaculation was accomplished using a manually controlled electroejaculator (Pulsator III; Lane Manufacturing, Denver, CO, USA) and a 75-mm rectal probe with three ventrally oriented electrodes. After collection, the semen samples were evaluated in the same way as the samples obtained by TM.

*Collection of semen samples by artificial vagina (**Paper IV**)*

Within two days after semen had been collected by TM (as described above), a second semen sample was collected from each bull with an AV. The bulls were previously trained and accustomed to serving an AV. The mount bulls were restrained in a short-sided breeding chute. The AV was prepared by filling the water jacket with hot water. Air was added to increase the pressure of the AV and the inside of the AV was lubricated with vaseline. After collection, the semen samples were evaluated in the same way as the samples obtained by TM.

Sperm morphology evaluation (Paper I-V)

*Evaluations performed in the semen lab (**Paper I, II, IV and V**)*

Sperm head morphology was studied in dry smears stained with carbol-fuchsine according to the method described by Williams (1920) and modified by Lagerlöf (1934). Five hundred spermatozoa were counted in each dry smear using phase contrast microscopy (x1000). The presence of proximal cytoplasmic droplets, abnormal acrosomes, detached heads and abnormalities of the midpiece and tail were recorded in wet-mount preparations of formol saline (4-5% aqueous solution of buffered formaldehyde) fixed spermatozoa. Two hundred spermatozoa were counted in each wet-mount preparation using phase-contrast microscopy (x1000). The abnormalities were classified according to a classification system developed by Bane (1961). Morphological abnormalities were recorded as the percentage of the total number of counted spermatozoa. The concentrations of sperm in samples collected by TM and AV were determined using a haemocytometer (Bane, 1952). Bulls with less than 15% proximal cytoplasmic droplets and less than 15% abnormal sperm heads were judged as having a mature semen picture (**Paper I**).

*Evaluations performed in the field (**Paper III**)*

The percentage live spermatozoa were determined by identifying spermatozoa that did not take up eosin stain in an eosin-nigrosin stained smear with bright field microscopy at 1000x magnification. Sperm morphology was determined by evaluating the number of abnormal spermatozoa per 100 sperm in an eosin-nigrosin stained smear with bright field microscopy at 1000X (Barth, 2000).

Macroscopic examination of hind limbs (Paper V)

Femorotibial, femoropatellar (stifle), tarsocrural, talocalcaneus, and proximal intertarsal joints from right and left hind limbs were examined postmortem. The joints were disarticulated and macroscopically examined for articular cartilage, synovial membrane/capsule, ligaments, menisci and subchondral bone lesions. The following lesions were noted: fraying, wear lines, erosion, and ulceration of the articular cartilage, osteochondrosis dissecans (OCD), cartilage retention, osteophytes and villiformation of the synovial membrane. Osteochondrosis (OC) was diagnosed when cartilage retention was found in a predilection site (Dutra *et al.,* 1999) and OCD when a cartilage rupture with a cartilage flap or loose osteochondral body could be seen. The lesions were recorded as unilateral or bilateral and graded as normal and with mild, moderate, severe or deformed osteoarthritis (OA).

Mild OA in the femorotibial and femoropatellar joints was characterized by superficial cartilage fraying of less than 30% of the articular cartilage, single erosion <1cm and superficial wear lines. Larger areas of cartilage fraying (>30% of the articular cartilage), multiple erosions, single ulceration <1cm, fragmentation of the intercondylar eminence of the tibia and villiformation of the synovial membrane were considered as moderate OA. Severe OA in the femorotibial and femoropatellar joints joint were characterized by single or multiple OCD, multiple ulcerations and single ulcer >1 cm. Deformed OA was identified when severe OA was present together with osteophytes.

Mild OA in the tarsocrural, talocalcaneus and proximal intertarsal joints was characterized by single erosion or single OCD. Multiple OCD and/or erosions in these joints were considered as moderate OA. Ulcers with denuded bone >0.5 cm were stated as severe OA.

Statistical analyses (Paper I-IV)

Statistical analyses were performed using the Minitab statistical software, release 13.31 (**Paper I and II**), release 14.2 (**Paper IV**) and Statistix software (Analytical software; Tallahassee, FL, USA) (**Paper III**). In **Paper I, II and IV** the data were analysed by using descriptive statistics. Correlations were made by simple correlation (**Paper I and II**). Paired T-tests were used in **Paper II and IV** to compare the sperm morphology parameters in semen samples collected by TM with those collected by CED and AV. P-values <0.05 were considered significant in **Paper I, II and IV**.

Results and General Discussion

Sexual maturity (Paper I)

According to the definition of sexual maturity given in materials and methods, less than half (48%) of the examined bulls were considered to have a mature semen picture. This is in accordance with a study on Canadian yearling beef bulls, where 42% of the bulls had a mature spermiogram (Arteaga *et al.,* 2001). The Canadian study was, however, based on evaluation of semen samples collected with electroejaculation (EEJ). Sperm concentration ≥250 million sperm per mL, motility ≥40% and at least 70% normal spermatozoa were included in their definition of a mature spermiogram. The definition we used in our study for sexual maturity (<15% proximal droplets and <15% abnormal heads) was adopted from a study by Einarsson *et al.* (1979) which used sperm morphology data from samples collected by cauda epididymal dissection (CED) postmortem from boars. This definition has, as mentioned earlier, also been used on Swedish yearling beef bulls and has according to the experience in our semen reference laboratory, proved indicative of the ability for normal reproduction in beef bulls (personal communication, Bane 1982). Several earlier studies (e.g. Einarsson *et al.,* 1979; Lunstra & Echternkamp, 1982; Johnson *et al.,* 1998; Amann *et al.,* 2000; Pant, 2000) have shown that the frequency of proximal droplets and abnormal heads decrease with age. Moreover Barth & Oko (1989) state: "bulls under 13 months of age had a higher incidence of sperm head abnormalities and proximal droplets than bulls 1.5 to 2 years." Both the persistence of proximal droplets as well as abnormal sperm heads is most probably influenced by factors that the sperm inherit from the testes (Rao, 1971) and thereby influenced by genetic factors (Bane, 1954). We found that proximal droplets positively correlated with abnormal heads, which is in line with data reported in dairy bulls (Rao, 1971). Lunstra & Echternkamp (1982) reported a rapid decrease in the percentage of spermatozoa with abnormal heads, as well as in the percentage of spermatozoa with proximal droplets in beef bulls during their first six weeks after onset of puberty, defined as the age at which an ejaculate was first obtained that contained a minimum of 50×10^6 total spermatozoa with at least 10% progressive motility. In the present study, the percentage of proximal droplets was highest in semen samples from 11-month-old bulls and then decreased with increasing age.

Arteaga and co-workers (2001) reported a frequency of 5.6% abnormal heads and 16.3% midpiece defects in Canadian yearling beef bulls. By comparison, the percentage of abnormal heads in our study was about three times as high (15.6%), whereas the percentage of midpiece defects only was 1.8%. This difference might, however, reflect differences in the sperm morphology classification system (Persson *et al.,* unpublished data). In Sweden, all sperm morphology evaluations of semen samples collected from bulls are currently performed solely at one semen reference laboratory by trained and very experienced technicians as described earlier. However, in many other countries (e.g. Canada) the semen evaluation is performed in the field by veterinary practitioners themselves (Palmer *et al.,* 2004).

For example, almost all sperm defects classified as sperm tail defects in the Swedish lab evaluation are instead classified as midpiece defects in the Canadian field study [e.g. midpiece reflexes (equal to simple bent tails), where the tail is curled around the midpiece (Barth & Oko 1989, Arteaga *et al.,* 2001)]. This probably explains the higher percentage of midpiece defects recorded in the Canadian field study compared to in our study. Different staining techniques used in the different protocols might also have contributed to the difference seen in the incidence of sperm head abnormalities. The very experienced technicians at our Swedish semen lab consider Eosin-Nigrosin, used in the Canadian field evaluation, to be a difficult stain for sperm morphology assessment, mainly due to the poor contrast between the spermatozoa and the background (Selin-Wretling, personal communication, 2006). This might explain the lower percentage of abnormal heads recorded in the Canadian study compared to in our study. It is also possible that the number of sperm abnormalities, recorded in the field evaluation protocol, are less sure due to a lower number of spermatozoa being counted (100 spermatozoa) compared to in the lab evaluation (200 and 500 spermatozoa).

In conclusion, Swedish beef sires are not fully sexually mature at 12 months of age. Our results indicate that only less than half of the studied Swedish yearling beef bulls evaluated at the performance testing station seems to have a mature semen picture at the time they are offered for breeding purposes. Due to a short Swedish pasture season, it is not always practically possible to postpone the breeding season and thereby let the bulls become older before use. Therefore, it is important to try to select bulls for breeding purposes with an early onset of puberty and a mature semen picture. Today, only growth rate is included in the breeding index for selection of a Swedish beef bull. The performance-tested beef bulls represent a group of bulls that have the highest impact on the breeding of beef cattle in Sweden. It is therefore essential to improve the breeding soundness evaluation scheme used today, at the performance testing station, to be able to improve the fertility potential of the bulls, at the time as they are offered for breeding purposes. Thus, it is essential to identify individuals with an early onset of puberty and a good semen quality. Even more important would be to exclude bulls with delayed puberty and poor semen quality.

Transrectal massage - general considerations (Paper II-IV)

Semen samples containing spermatozoa were obtained by transrectal massage (TM) from a majority of the bulls (**Paper II**: 90.4%, **Paper III**: 80.9%), see Table 1 for semen collection data in detail. These success rates are similar (or somewhat lower) to other studies involving older (>15 months) bulls (Goodwin, 1970; Palmer *et al.,* 2004). Practitioners can, without any prior training, easily learn the technique, from a written instruction, and successfully collect semen from bulls in the field (Persson & Söderquist, unpublished). Our own experience from the field is that it is easier and quicker to collect semen with TM in older bulls compared to in yearling bulls. According to our experience it is often, however, not possible to obtain semen sample if the bull has not responded at all after 5 minutes of massaging. It has been observed that TM seems to act as a distraction to the bulls and tends to keep them quiet during collection (Parsonson *et al.,* 1971). None of the yearling bulls in the present study displayed behaviour during or after the procedure that would indicate compromised animal welfare.

Table 1. Semen collection data and semen characteristics in bulls collected by transrectal massage (TM), cauda epididymal dissection (CED), electroejaculation (EEJ) and artificial vagina (AV) in three different papers (**II-IV**).

Paper	II	II	III	III	IV	IV
Semen collection technique	TM	CED	TM	EEJ	TM	AV
Number of ejaculates	47	23	68	82	14	14
Semen samples collected (%)	90	100	81	100	-	-
Sperm motility (%)	43	-	50	60	57	62
Sperm conc. (x10^6 sperm/ml)	202	-	320	724	61	1425
Abnormal heads (%)	16	16	1	1	12	13
Detached heads (%)	5	2	3	1	6	3
Abnormal acrosomes (%)	0.3	0.5	-	-	0.2	0.5
Proximal cytopl. droplets (%)	4	13	1	1	1	2
Abnormal midpieces (%)	1	2	5	3	1	1
Abnormal tails (%)	5	14	-	-	3	3

– = missing data

Sperm volume (**II** and **IV**) and concentration (**II-IV**) were lower in the semen samples collected by TM, than in samples collected by artificial vagina (AV) or EEJ. Attempts to collect maximum volumes were not made, but unintentional stimulation of the seminal vesicles during TM might have resulted in increased emission of seminal fluid and thus collection of samples with lower sperm concentration.

Reportedly, semen samples collected by TM are more heterogeneous and inconsistent than semen samples collected using EEJ (Chenoweth, 2001; Wolfe, 2001). One reason for this might be that semen collected by TM probably often originates from the ampullae rather than from the caudae epididymes. These findings are also in line with the results of our studies, where we found a wide range in both sperm concentration and motility of samples collected by TM. Sperm motility was on average lower in samples collected by TM in **Paper II, III** and **IV** (43%, 50%, 57%; respectively) compared with those collected with EEJ (60%) and AV (62%). The sub-optimal weather conditions during semen collection in **Paper II** and **III** might have contributed to the lower sperm motility seen in some of the semen samples despite all efforts to keep the samples warm. Another explanation for the lower sperm motility could be that semen often dribbles slowly from the penis resulting in more prolonged exposure to air and ambient temperature on the surface of the semen collection funnel.

Sperm morphology parameters did not differ between the different semen collection methods in **Paper III** (TM vs. EEJ) and **IV** (TM vs. AV). In **Paper II** (TM vs. CED postmortem), the frequency of all sperm morphology parameters differed statistically, except for the frequency of abnormal heads and abnormal acrosomes, which were equal in the semen samples for the two semen collection methods (see below).

In conclusion, transrectal massage is a useful technique that can be used to collect semen from yearling (and older) beef bulls in situations where use of an artificial vagina or electroejaculation is not possible, thus enabling a complete BBSE on a routine basis. Semen collection by TM does not seem to harm the bull, but it appears that well-handled, calm bulls have a higher success rate of semen emission than fractious bulls. The TM technique does not seem to affect sperm morphology in the collected semen samples. However, reduction in volume, sperm concentration and motility would need to be considered in semen quality classification, especially if semen is collected outdoors under suboptimal conditions.

Transrectal massage vs. cauda epididymal dissection postmortem (Paper II)

Samples from thirty of the bulls (64%) had a sperm motility of >30% and semen samples from 21 (45%) of the bulls had a sperm concentration of >100 x 10^6 spermatozoa/mL. Semen samples collected by TM had a lower percentage of proximal droplets (p=0.000), abnormal tails (p=0.015) and abnormal midpieces (p=0.045), than in the CED samples, whereas the percentage of detached heads was higher in the TM samples (p=0.016) than in the CED samples. However, the percentage of abnormal heads and abnormal acrosomes did not differ significantly between the two methods of semen collection. The percentage of proximal droplets and abnormal heads in the caudae epididymal samples correlated strongly (p=0.000) to the corresponding defects in the TM samples, whereas the percentage of detached heads and abnormal tails in the caudae epididymal samples did not correlate to the corresponding defects in the TM samples.

Rao (1971) reported that dairy bulls (2-12 years of age) with a history of impaired fertility had a much higher percentage of proximal droplets in the cauda than in the ampullae. Almost half the bulls in our study were not fully sexually mature, which might be one explanation for the elevated average levels of proximal droplets found in the cauda. This is to some extent supported by a pilot study (Persson & Söderquist, unpublished data) where semen was collected from five yearling (approx. 15 months old) beef bulls, by repeated TM, twice a day during two consecutive days. After slaughter, the day after the last TM collection, the genital organs were retrieved and semen was collected from the ampullae (by flushing with saline solution) and by CED for further sperm morphological assessment. Theoretically one could speculate that the proximal droplets are shed from the spermatozoa on their way from the caudae to the ampullae or gradually disappear if spermatozoa are stored or accumulated in the ampullae for a longer period due to senescent processes changing the morphological appearance of the spermatozoa. The latter hypothesis is to some extent supported by the higher incidence of detached heads (see below) found in the TM samples compared to in the CED samples. A repeated TM would consequently result in a lower incidence of proximal droplets. In our pilot study we found that bulls with elevated levels of proximal droplets (>15%) in the CED samples had lower levels in the ampullae and even lower in the TM samples, whereas bulls with low levels of proximal droplets in the CED samples had the same low levels in the ampullae and in the TM samples. This is in agreement with the statement by Rao (1971): "The migration of the cytoplasmic droplets was almost complete in normal bulls by the time sperm reached the collection site in region D (i.e. distal part of caput), whereas in pathological bulls this process continued in later regions." However, there was no difference in the level of proximal droplets between the repeated collections within the same bull, so the reason for the difference found in the incidence of proximal droplets in TM compared to CED collected samples still is not fully understood.

The higher percentages of abnormal tails (mainly simple bent tails) found in the postmortem samples in **Paper II** may be an artefact, due to cold shock of the sperm in the cauda (Barth & Oko 1989), since the testicles postmortem were placed on crushed ice for transportation from the slaughterhouse to the laboratory. Furthermore, the percentages of detached heads were higher in the massage samples than in the postmortem samples. The bulls in this study had never been used for breeding purposes prior to the study and thus the increased incidence of detached heads might be due to accumulation of senescent sperm in the ampullae. Accumulation of spermatozoa in the epididymis and/or the ampullae has been described in some bulls and is associated with elevated levels of detached heads in the ejaculate (Barth & Oko 1989). Interestingly, no difference in the percentage of abnormal sperm heads was found in our study between the samples collected by transrectal massage and those collected by caudal epididymal dissection postmortem.

According to the recommendations for semen samples from beef bulls, routinely used at our reference semen lab, none of the percentages of the sperm abnormalities must exceed 15% in the sample, to be judged as approved (Bane, personal communication, 1982). Based on this definition and using the data for the bulls which had samples of semen collected by both methods, 43% of the samples collected by TM and 39% of the samples collected by CED postmortem were judged to have satisfactory sperm morphology, respectively. This is in accordance with **Paper I**, and also with a study on Canadian yearling beef bulls, where 42% of the yearling beef bulls passed as satisfactory breeders (Arteaga *et al.,* 2001). The Canadian study was based on evaluation of semen samples collected by EEJ.

Transrectal massage vs. electroejaculation (Paper III)

Electroejaculation was more likely to result in successful semen collection (100%) compared with TM (80%). Semen was successfully collected by EEJ from all bulls in which TM had failed. Time required for obtaining a semen sample and semen characteristics did not differ between samples collected by EEJ or by EEJ after failed TM attempt. For both EEJ and TM, the time required to prepare to collect the next bull was approximately the same and limited by the time it took to get the next bull into the chute. However, the time required to obtain a semen sample with TM was greater ($p<0.001$) than with EEJ. This means that EEJ was a more reliable method to obtain semen because it required less time and had 100% efficacy. However, these bulls were not accustomed to handling and they were clearly more excited than the bulls included in **Paper II** and **IV**. It appears that handling stress is a major limiting factor for successful semen collection by TM in beef bulls (Palmer *et al.,* 2004). Semen samples collected by TM had lower sperm concentration ($p<0.001$), percent motile ($p<0.05$) and live sperm ($p<0.001$) than samples collected by EEJ, but sperm morphology did not differ (Table 1). The prolonged exposure of sperm to air, temperature and environment in the distal preputial cavity might reflect the low motility and viability in the semen samples collected by TM. On the other hand, possible temperature and preputial fluid effects on sperm do not seem to be so detrimental as to affect sperm morphology. When comparing TM to EEJ it is also important to remember that many factors may affect concentration and volume in semen samples collected by EEJ, making these characteristics unreliable for evaluation of semen quality (Barth, 2000).

Transrectal massage vs. artificial vagina (Paper IV)

Interestingly, when comparing semen collected by AV or TM, no difference could be seen in the percentage of motile spermatozoa or in the incidence of sperm abnormalities assessed. According to the previously described definition (none of the assessed sperm morphology parameters must exceed 15%), 79% of bulls collected by TM and 71% of bulls collected with an AV were judged to have satisfactory sperm morphology. Semen samples from all (14/14) bulls collected by TM and AV had ≥30% motility and in 18% (2/11) of the TM samples and in 100% of the AV samples, the concentration was $>100 \times 10^6$ spermatozoa/ml. Hence, sperm concentration ($p=0.000$) as well as volume ($p=0.001$) were higher in samples obtained by AV than in the TM samples (Table 1). However, attempts to obtain maximum volumes with TM were not made in this study. The reason that the percentage of motile sperm did not differ between TM and AV may be related to more optimal conditions in general, compared to collection in the field, and to the indoor temperature during semen collection. As mentioned earlier, semen collected by TM often dribbles slowly from the penis resulting in more prolonged exposure to ambient temperature, which seems to affect sperm motility and viability, but not sperm morphology.

Joint lesions (Paper V)

A bull with joint lesions may have difficulties in completing the mounting act due to pain caused by cartilage ulcerations, subchondral bone sclerosis with micro fractures and/or synovial membrane inflammation. Most of the bulls (73%) with impaired fertility had severe or deformed osteoarthritis (OA) with cartilage loss and sclerotic denuded subchondral bone (Figure 1). The control bulls, although older, had mild or moderate OA or no lesions at all. The localisation and nature of the joint lesions in the present study are consistent with other descriptions of OC in cattle (Reiland et al., 1978).

Figure 1. Distal femur from a Charolais bull. Cartilage ulcers (arrows) with denuded bone and osteophytes in the lateral trochlear ridge of distal femur. Deformed osteoarthritis.

Almost all (89%) of the bulls with impaired fertility as well as the control bulls with normal fertility (91%) had lesions in at least one joint. Most of the bulls had lesions in the femorotibial joint (77% for infertile bulls, 100% for control bulls) with cartilage fraying of the proximal tibial plateau, and almost all of them were bilateral (83% and 80%, respectively). However, most of the lesions in the femorotibial joint were mild or moderate with only superficial cartilage changes without denuded bone or osteochondrosis dissecans (OCD), probably of a minor clinical importance.

The most common site of lesions in the femoropatellar joint with moderate, severe to deformed OA was the lateral ridge of the femoral trochlea, often characterized by cartilage ulcers with denuded bone. This is one predilection site for OC in cattle, also reported by others in previous studies (Reiland et al., 1978; Weisbrode et al., 1982; Trostle et al., 1997; Tryon & Farrow 1999).

Most of the lesions in the lateral femoral trochlea were bilateral (14/20 bulls with impaired fertility and 4/6 of control bulls) and more severe in the bulls with impaired fertility. The conformation of these lesions renders them likely to produce joint pain and hence clinical symptoms such as synovial effusion, difficulty in mounting and hind limb lameness.

We believe that the presence of bilateral joint lesions will make it more difficult for the farmer to observe clinical gait asymmetries. None of the bulls in this study were noted to have gait asymmetries although most of the bulls had postmortem lesions compatible with severe OA. This is in accordance with previous observations (Baxter, 1991). The bulls had not been examined for lameness with joint palpation including flexion test and radiological examination, which is always done in horses. This thorough joint examination is not possible in beef bulls that are not used to handling. Hence, joint lesions with synovial effusion and hind limb lameness may not be recognized as a cause of infertility in the beef sires. Moreover, the most severe lesions (lateral ridge of the femoral trochlea) in this study were found at a proximal position of the trochlea in the femoropatellar joint, where there is no weight bearing articular cartilage during normal movements. However, the patella glides over the femoral trochlea when the bull mounts, and this can cause joint pain and prevent the bull from completing the mating.

Osteochondrosis is probably one of the most common causes of OA in the stifle of the beef bull. A postmortem study of yearling beef bulls (Dutra et al., 1999) showed that almost 100% had lesions compatible with OC latens or manifesta. Factors such as heavy body weight and trauma to the joint tissue, due to behaviour of the bull (frequent mounting), can influence the dynamics of the joint. A heavy load applied to cartilage with OC manifesta can cause development of an OCD and subsequent OA (for review see; Ytrehus et al., 2007).

The control bulls in the present study were older than the infertile bulls, a consequence of their having served successfully in herds; however, fewer and milder joint lesions were recorded in these control bulls. The infertile bulls were culled at a younger age and were noted to have more and severer joint lesions. This further strengthens the conclusion that the infertile bulls in this study had joint lesions that contributed to their reproduction problems, and subsequent culling.

Furthermore, the bulls culled due to infertility, but with a satisfactory semen picture (13/26) had joint lesions, mostly representing severe or deformed OA (77%). Consequently, the most likely cause of infertility in these 13 bulls was mounting problems as a result of hind limb leg weakness.

We conclude that joint lesions, often compatible with OC in the hind limbs of beef bulls, can be a contributing factor to impaired fertility in the beef herd and should be taken into consideration when conducting bull breeding soundness evaluations.

General conclusions

- Less than half of the studied Swedish yearling beef bulls, evaluated at the performance testing station, had a mature semen picture at the time they were offered for breeding purposes at about 12 months of age.

- Transrectal massage is a useful technique that can be used to collect semen from yearling (and older) beef bulls in situations where use of an artificial vagina or electroejaculation is not possible, thus enabling a complete bull breeding soundness evaluation including semen evaluation.

- Joint lesions should always be taken into consideration as a contributory cause of reproductive failure in beef sires with or without symptoms of lameness.

Recommendations for beef breeding

– based on our results

1. Beef bulls are not always sexual mature at twelve months of age and should not be used for breeding purposes at this young age.

2. A complete Bull Breeding Soundness Evaluation (BBSE) should be performed on all beef sires before purchase, and - annually - before the breeding season. The complete BBSE should include a general health check with special focus on the musculoskeletal system, including gait asymmetries and/or mounting problems, and a special andrological examination, including semen evaluation.

3. Semen samples can be collected by transrectal massage in most beef bulls in the field and a sperm evaluation should be included in the BBSE protocol.

4.
Future research

- **Find out when Swedish beef bulls are fully sexually mature.**

By repeated semen collections with transrectal massage (TM), I would like to follow a group of yearling beef bulls from 12 to 16 months of age to evaluate their sperm morphology with increasing age.

- **Improve the bull breeding soundness evaluation (BBSE) protocol for field conditions in Sweden.**

To apply the results, achieved in this thesis, I would like to develop a new BBSE protocol for Swedish field conditions. This protocol should include a general health check, a special andrological examination and semen collection by TM. It should also include a simplified protocol for semen evaluation in the field resembling the Canadian BBSE protocol that is in use. I would like to evaluate the use of such a BBSE protocol for Swedish beef bulls by letting practitioners examine beef sires prior to the breeding season and exclude unsatisfactory bulls and then compare the calf output in the herds with evaluated, satisfactory bulls with herds with non-evaluated bulls.

- **Develop better methods for diagnosing joint disorder of beef bulls *in vivo* to include examination of joints in the BBSE.**

A pilot postmortem study (Persson *et al.*, unpublished data) showed that magnetic resonance image (MRI) is a possible method for diagnosis of OC manifesta as well as OCD in beef bulls. A longitudinal, study, including a clinical and radiological examination as well as an MRI examination of a group of young beef bulls, introduced to their first breeding season should be performed. This study could evaluate which type of joint lesions were associated with mounting problems and hence infertility in the herd. This would also clarify whether bilateral joint lesions of hind limbs do cause lameness in the bull.

- **Study aetiological factors of osteochondrosis in Swedish beef bulls.**

It would be of great importance to evaluate aetiological factors such as diet, growth rate, body weight, flooring, exercise and beef breed in relation to the development of OC manifesta, OCD and OA. This knowledge would provide data for better management recommendations to the beef breeders resulting in healthier bulls.

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Populärvetenskaplig sammanfattning

Sveriges nötkreatur kan förenklat delas upp i två olika grupper, köttdjur respektive mjölkdjur. Mjölkkorna hålls, som namnet antyder, framför allt för att de ska producera mjölk, medan köttkorna, eller de s.k. dikorna, ska producera kött i form av kalvar. Det övergripande produktionsmålet i en dikobesättning är - en levande kalv per ko och år. För att uppnå detta mål är det viktigt att djuren har en hög fruktsamhet. Det är särskilt viktigt att avelstjuren är fruktsam och hållbar, eftersom de flesta köttdjursuppfödare använder en tjur för fribetäckning i sin besättning. I en mjölkkobesättning används däremot nästan uteslutande semin. För att den fribetäckande tjuren ska ha en god fruktsamhet måste han vara fullt könsmogen och producera sperma av god kvalitet. Dessutom måste han ha ett bra skelett, friska leder och klövar och en välutvecklad muskulatur för att klara av att upprepade gånger hoppa upp och betäcka hondjuren.

Vi har i en studie visat att tolv månader gamla, blivande köttrastjurar avsedda för avel inte är könsmogna i samma utsträckning som jämnåriga mjölkrastjurar, trots att köttrastjurarna förväntas påbörja sin tjänstgöring i besättningen vid den åldern.

För att undersöka en tjurs potentiella fruktsamhet bör man vid köp och försäljning samt årligen inför varje betäckningssäsong göra en allmän hälsoundersökning och en andrologisk undersökning av tjuren, dvs undersöka tjurens könsorgan. Även en spermaprovtagning bör ingå i denna undersökning. Idag säljs tyvärr de flesta av de omkring tusen köttrastjurar, som förmedlas till avel i Sverige varje år, utan en föregående veterinärbesiktning. Ett åttiotal ettåriga, blivande avelstjurar säljs varje år efter sex månaders s.k. individprövning på Sveriges enda prövningsstation för köttjurar. På Gismestads tjurprövningsstation utanför Linköping prövas tjurarna med avseende på tillväxtegenskaper, mentalitet och exteriör. Stationsveterinären utför också en allmän hälsoundersökning och en andrologisk undersökning där han känner på testiklar, bitestiklar, pung och sädesledare, inspekterar penis och förhud samt mäter pungomkretsen. Pungomkretsens storlek är direkt kopplad till testiklarnas vikt, som i sin tur är direkt kopplad till spermieproduktionsförmågan hos testiklarna. Pungomkretsen är således ett indirekt mått på tjurens förmåga att producera sperma. Tyvärr finns det ingen möjlighet att samla sperma på dessa tjurar, och vid ett års ålder säljs de på livdjursauktionen, ofta för betydande summor, utan att köparen vet något alls om den inköpta tjurens spermieproduktionsförmåga eller spermans kvalitet.

Det vanligaste sättet att samla sperma på mjölkrastjurar är med hjälp av en s.k. artificiell vagina på en tjurstation. Det går till så att tjuren får bestiga en låtsasko, en s.k. fantom, eller en annan tjur, varvid sperman samlas upp i den artificiella vaginan. Spermasamling med hjälp av en artificiell vagina är svårt, och ibland också farligt att utföra på otränade köttrastjurar under fältmässiga förhållanden. Sperma från köttrastjurar samlas i fält framför allt med hjälp av så kallad elektroejakulation där en elektrisk stav kopplad till ett batteri förs in i ändtarmen på tjuren. Elektriska impulser från staven leder till att tjuren får utlösning. Denna metod är av djurskyddsskäl förbjuden i Sverige. I Sverige har vi därför framför allt fått förlita oss på spermaprover tagna från bitestikelsvansen (= spermiernas lagringsplats) på slaktade djur. Denna metod har av förståeliga skäl sina begränsningar. Vi har således hittills inte haft möjlighet att samla sperma på köttrastjurar i fält, vilket förstås är begränsande då man på ett bättre sätt skulle vilja försöka varudeklarera tjurens potentiella fruktsamhet. De individprövade tjurarna är de tjurar som har störst betydelse för den inhemska aveln. Genom att utveckla och förbättra ettårsbesiktningen av dessa tjurar, ffa genom att samla och bedöma deras sperma, skulle man kunna upptäcka tjurar med sen könsmognad och dålig spermakvalitet och därmed utesluta dem ur aveln.

Spermasamling med hjälp av s.k. rektal massage finns beskriven på människa, elefant och nötkreatur. Genom att, via ändtarmen (rektum), massera delar av de inre könsorganen stimulerar man tjuren till utlösning. Det finns ett fåtal studier där man jämfört spermaprov erhållna med hjälp av rektal massage med prov samlade med elektroejakulation. Vidare saknas undersökningar där man jämfört spermaprov erhållna med massage med prov samlade med hjälp av artificiell vagina. Det finns heller ingen undersökning som visat att man med hjälp av rektal massage kan samla sperma på tolv månader gamla tjurar. Vi har i tre olika studier samlat sperma på tjurar med rektal massage och jämfört spermiernas utseende och form i dessa prover med prover samlade från bitestikelsvansen, samt med prover samlade med elektroejakulation respektive med artificiell vagina. I de flesta fall har vi fått en god samstämmighet mellan metoderna, framför allt mellan prover samlade med massage och med artificiell vagina, som ofta anses var den spermasamlingsmetod som närmast liknar situationen vid naturlig parning. Vi har också kunnat visa att man enkelt och snabbt kan samla sperma från ca tolv månader gamla köttrastjurar med hjälp av rektal massage. Det är emellertid viktigt att komma ihåg att man måste bedöma den ofta lägre volymen, spermiekoncentrationen och -motiliteten med en viss försiktighet i prover samlade med hjälp av massage.

En fribetäckande tjur måste kunna klara av att fullfölja upphopp och betäckning och för det krävs bl a friska leder i bakbenen. Vi har obducerat knä- och hasleder från ett trettiotal avelstjurar av köttras. Tjurarna slaktades på grund av ett dåligt dräktighetsresultat i besättningen. Ingen av tjurarna hade visat några tecken på hälta eller annan rörelsestörning. En majoritet av tjurarna visade sig ha kraftiga dubbelsidiga ledskador i framför allt bakbenets knäleder. De flesta av skadorna berodde på en ledsjukdom som kallas osteokondros. Osteokondros är en sjukdom som drabbar tillväxtbrosket i det unga djurets skelett. Det unga djurets skelett består till stor del av tillväxtbrosk som ska omvandlas till skelett. Om denna benbildning störs kommer inget skelett att bildas och de delar av skelettet som drabbas kommer att bli sköra och svaga. Skadorna utvecklas framför allt i ändarna på benen, nära lederna. Sjukdomen uppkommer på grund av en lokal störning av blodförsörjningen i tillväxtbrosket, vilket leder till syrebrist som i sin tur leder till vävnadsdöd av brosket. Vad detta beror på är inte helt klarlagt, men både ärftliga faktorer och miljöfaktorer har diskuterats. Osteokondros är vanligast på handjur och det är framför allt de tyngre raserna som drabbas. Man anser att hög tillväxt med hög kroppsvikt bidrar till osteokondros. Brist på motion och hårda golv är andra bidragande faktorer, som kan ge en ökad belastning i leden, där det skadade brosket finns. Vi tror att tjurarna pga. ledskadorna hade så pass ont i benen, att de därför inte klarade av att betäcka hondjuren. Eftersom skadorna var dubbelsidiga var det sannolikt svårt att se någon hälta. För att ytterligare styrka vår tes, obducerade vi bakbenen från elva normalslaktade tjurar, som hade haft ett gott dräktighetsresultat. Även dessa tjurar hade ledskador, men skadorna var lindrigare, trots att dessa kontrolltjurar var äldre än tjurarna med dåligt dräktighetsresultat.

Sammanfattningsvis kan vi konstatera att svenska köttrastjurar inte är fullt könsmogna vid tolv månaders ålder. Tjuren bör ha blivit några månader äldre innan de sätts in i avel. För att i möjligaste mån försäkra sig om att man köper in en könsmogen, fruktsam tjur bör ett spermaprov ingå i veterinärbesiktningen. Samling av sperma på köttrastjurar i fält kan relativt enkelt och säkert utföras med hjälp av rektal massage. Våra resultat visar att det går bra att samla sperma prov även från ettåriga tjurar. Vi kan också konstatera att ledskador kan vara en bidragande orsak till ett dåligt dräktighetsresultat, varför det är viktigt att även undersöka rörelseapparaten då man besiktar tjuren. Om möjligt bör man även studera tjuren i rörelse.

Sammanfattande rekommendationer

– som bygger på våra och andras forskningsresultat

- Avelstjurar bör vara äldre (några månader) än tolv månader då de sätts in i avel.
- Avelstjurar bör veterinärbesiktas före köp/försäljning och årligen före varje betäckningssäsong, samt om man misstänker att något är fel på tjuren. I besiktningen bör följande ingå:
  - Genomgång av tjurens sjukdomshistoria och bakgrund
  - Allmän klinisk undersökning, inklusive en noggrann genomgång av ben och leder, samt en kontroll av tjurens rörelser.
  - Speciell andrologisk undersökning av könsorganen, inklusive mätning av pungomkrets, samt spermasamling och – bedömning.
- Spermasamling på köttrastjurar i fält kan med fördel utföras, även på ettåriga tjurar, med hjälp av rektal massage.
- För att minska uppkomsten av ledskador bör följande eftersträvas:
  - Avelstjurar bör inte hållas uppbundna under stallsäsongen. De bör inte hållas på hårda golv eller på spalt utan bör gå i box eller i lösdrift med mjukt underlag på en del av golvytan.
  - Avelstjurar bör inte vara för tunga. Tillväxten bör begränsas så att inte slutvikten blir för hög. De flesta tjurar kan behöva bantas inför betäckningssäsongen.
  - Avelstjurar bör få motion. Om tjuren har stått i en mindre box under stallsäsongen bör han rörelsetränas samt successivt vänjas vid hondjursflocken i samband med betäckningssäsongens början.
  - En ungtjur bör inte släppas till för många hondjur (max 15) första betäckningssäsongen.