Scientific Advisory Committee on Animal Health and Welfare

Task

Currently in Ireland, th**e** practice of electroejaculation (EEJ) is being used to facilitate the collection of semen samples from species used in farm animal production in order to determine their soundness for breeding purposes. Both registered veterinary practitioners and non-registered persons are providing this service. It is the Department's understanding that the practice is regulated in the UK, being confined to veterinary practitioners.

The purpose of this task is to establish whether the practice of electroejaculation involves "interference with the sensitive tissue of an animal" and if so, evaluate the extent to which this procedure causes pain & discomfort. The remit of this task is to also investigate if anaesthesia and/or analgesia can be successfully used to ameliorate any pain or discomfort that may arise.

Key words: Semen collection, Pain, Analgesia, Anaesthesia, Farm Animal, Animal Welfare, Veterinary.

Background

Description of electro-ejaculation (EEJ)

Electro-ejaculation (EEJ) is a procedure used to collect semen by electrically stimulating the pelvic nerves and surrounding tissues. It has been used as method of inducing ejaculation in domestic animals since 1936 (Palmer 2005). The procedure involves the insertion of an electric probe into the rectum to gain contact with the intrapelvic area (2). Ejaculation is achieved by applying a series of short, low-voltage pulses of electrical current to the pelvic nerves, which stimulates the smooth muscles of the ampullae and vas deferens, inducing the ejaculatory response (1,4). The equipment used for the procedure includes rectal probes of variable sizes and an electrical current generator.

In livestock production, semen collection is a procedure used as part of breeding soundness examination, and to a lesser extent for disease diagnoses e.g. *Brucella Ovis* in Rams (Stafford, 1995). The breeding soundness examination is an important management tool used to assess a male's fertility potential. According to the Merck Veterinary Manual "*The breeding soundness examination involves a complete and systematic evaluation of the reproductive potential of a given male, including mating ability and libido, general physical examination and inspection of the genital organs, and assessment of sperm production and quality*" (5). There are four methods of semen collection: vaginal aspiration from a recently bred female, use of an artificial vagina (including internal artificial vagina), transrectal massage and EEJ (Palmer 2005).

There is no systematic recording of the frequency of EEJ in Ireland. It is mainly used on-farm to collect semen in bulls pre-sale, as part of a breeding soundness examination and not used at AI Centres. In Ireland, there are currently no regulatory requirements for the training of EEJ operators, no technical specification of the EEJ equipment and no code of practice to safeguard the welfare of livestock during EEJ. Regarding EEJ operators, the procedure is carried out by both private veterinary practitioners and non-veterinarians. Organisations such as the Cattle Association of Veterinary Ireland, British Cattle Veterinary Association and Embryonics (UK) provide accredited training courses for veterinary practitioners on the use of EEJ in bulls.

Scientific Evidence

A number of studies have been conducted to assess pain response during EEJ, with and without the use of anaesthesia and analgesia. Table 1 provides a summary of the experimental studies referred to in this review. In addition, Stafford (1986) and Palmer (2005) have published reviews of pain associated with EEJ.

This review presents a summary of operational factors effecting pain responses associated with EEJ, based on scientific evidence and considers regulatory frameworks from other European Member States (Table 2).

The duration, intensity and number of animals affected can be used to assess animal welfare. The probe type and pattern of electrical stimulation determine the area of tissue affected and the duration and intensity of electrical stimulation required to achieve ejaculation, respectively.

Probe type

Rectal probes have been developed over the years and conventional ring probes, where electrical current is emitted circumferentially, are being replaced in favour of ventral probes, which deliver the electrical current in a more targeted way (Stafford 1986). Older probes, with ring electrodes surrounding the barrel of the probe, had adverse effects. For example stimulating nerves dorsal to the rectum that supply the skeletal muscles of the back and hindlimbs resulted in muscle haemorrhage and bruising and animals remained stiff of gait for a number of days after EEJ (Stafford et al 1995).

There are two types of ventral probes: segmented and non-segmented. Non-segmented ventral probes, like conventional ring probes, can stimulate non-target tissues unnecessarily. In contrast, segmented probes allow operators to reduce the stimulation of non-target tissue in the intrapelvic area (Etson et al 2004). These devices have three electrode segments, which stimulate EEJ in either two or three phases (Etson et al 2004):

i. 3 short caudal segments, to induce penile protrusion,
ii. 3 short middle segments to induce semen emission
iii. 2 short cranial segments, which are generally only used when EEJ is not elicited by step (ii)

Younger bulls tend to only need activation of the caudal segments, whereby older bulls may require activation of the cranial segments (Palmer 2005a).

Rectal probes of 65mm-75mm in diameter and 360mm in length are generally used for EEJ in bulls (Palmer 2005, Whitlock et al 2013, Etson et al 2004, Falk et al 2001, Pagliosa et al 2015). Larger probes, up to 90mm in diameter may be required for older bulls to allow better contact with rectal mucosa (Barth 1997 cited in Palmer 2005).

Pattern of Electrical Stimulation

The intensity and duration of electrical stimulation is an important consideration for animal welfare. Palmer (2005) advocates EEJ equipment that uses sine wave pulse at frequencies of 20-30 cycles wave form of electrical stimulation, because less electrical stimulation is required to achieve EEJ compared to other wave forms (Furman et al 1975 and Salisbury et al 1978). Bull probes generally give a maximum voltage of 13V -16V (Whitlock et al 2012, Palmer 2005), with a maximum current of 900Ma. Most bulls ejaculate with an electrical impulse of less than <8 or 9V (8, Palmer 2005a).

Experiments on EEJ have used different patterns of electrical stimulus and rest periods. Some used 1-2 or 2-3 seconds of electrical stimulus and a short rest interval of 0.5-1 seconds (Palmer 2005a, Pagliosa et al 2015), whereas others applied equal length stimulus (2 seconds) and rest periods (2 seconds) (Mosure et al 1998). According to Stafford (1995), the stimulation should be almost continuous, as a rest period for even 1 second could impede erection in bulls. In the studies reviewed, which used various stimulus and rest periods, the time to semen emission ranged from approximately 90 seconds (Pagliosa et al 2015) to 214 seconds (Whitlock et al 2012). One study of 137 bulls showed that successful semen collection was achieved at an average of 100 seconds of stimulus cycles (Palmer et al 2005b).

Pain Responses Associated With EEJ

A number of studies have been conducted to investigate the effects of EEJ on pain indicators such as vocalisation, blood cortisol, and heart rate and have been summarised by Palmer (2005).

Animal behaviour

Vocalisation is considered a more accurate indicator of pain compared to other behavioural responses (Falk et al 2001). It is an immediate response to pain and can therefore indicate the stage of a procedure which is most painful. In addition, vocalisation is considered to be a more reliable indicator of acute pain than physiological measurements such as heart rate or blood cortisol, because the latter react to moderate levels of stress such as handling and restraint (Watts et al 1999).

Grandin (2010) refers to unpublished data (BD Voisinet and T Grandin 1997), comparing vocalisation response in bulls to restraint only, and restraint with high and low voltage EEJ (Table 3). Similarly, Whitlock et al (2012) showed significantly more vocalisations in bulls during EEJ than during a control (no) treatment (56% vs 0%), supporting other studies in the conclusion that EEJ is a painful procedure. Falk et al (2001) designed a scoring system for recording vocalisations in bulls undergoing EEJ with and without epidural anaesthesia. Whilst they showed no significant treatment differences, Palmer (2005a), showed that by modifying the scoring system, 25% of bulls without a lidocaine epidural treatment vocalised moderately to severely during EEJ when compared with 0% of bulls treated with a lidocaine epidural. Etson et al’s study (2004) reported that 1 in 10 bulls mildly vocalised in response to EEJ with a segmented probe. Palmer (2005a) suggests that lack of vocalisation does not necessarily mean lack of pain and that the differences in vocalisations recorded between Falk’s and Etson’s study may be associated with EEJ technique (Palmer 2005a).

In a study of vocalisations during EEJ in generally anaesthetised Pampas deer, it was found that the vocalisations related to the strength of the voltage applied during the EEJ process (Fumagilli et al 2015). This study also showed that younger males, vocalised more during the EEJ procedure than adult male Pampas deer, indicating that age may influence the pain response to EEJ (Fumagilli et al 2015). A study was carried out in rams showing a significant relationship between vocalisation and pain during EEJ (Damian et al 2011).

Choice, preference or aversion tests provide another behavioural assessment of pain. These tests use avoidance learning to evaluate an animal’s response to the potential of a painful experience. In EEJ, the level of aversion can be influenced by restraint techniques and probe type (Stafford 1995). Moving rams along a race for EEJ produced a degree of aversion that was significantly higher than movement through a race for no procedure but was slightly less than that associated with part-shearing (Stafford, 1995). Similarly, in bulls, when the procedure was carried out frequently (2-3 times a week for 6 weeks), the study failed to show aversion to handlers or restraint facilities (Barth et al 1994).

Pagliosa et al (2015) evaluated behavioural indicators of pain, discomfort and ataxia in eight 30 month old Nellore bulls (Bos tauras indicus) during EEJ, with and without an epidural anaesthetic. The control group (no anaesthetic) showed severe signs of pain and discomfort compared to bulls administered with epidural anaesthetic (either lidocaine, xylazine or xylazine plus hyaluronidase). The anaesthetised bulls showed mild signs of pain during the EEJ procedure.

Physiological changes in response to pain during EEJ

Relatively few studies have been carried out to evaluate the role of blood cortisol as a pain indicator during EEJ. However blood cortisol may be an unreliable indicator of pain during EEJ, because it is elicited by activities requiring energy mobilisation (gluconeogenesis) including normal sexual activity (e.g. Moberg 2000; Szechtman et al 1974) and external situational factors, such as handling.

Cortisol concentrations tended to be higher in bulls undergoing EEJ without a pain reduction treatment such as a lidocaine epidural (Falk et al 2001), supporting its relationship with the bulls pain response.

In a study of 18 rams, that had previously undergone EEJ, the effect of EEJ on blood cortisol was evaluated. Two treatments were applied: rectal probe insertion only and rectal probe insertion plus EEJ (Orihuela et al 2009). A control group was also incorporated into the study, where no treatment was applied. Results showed that blood cortisol was significantly increased in the group of rams that underwent EEJ than the group that were treated with probe insertion only. The control group and the probe-only group showed no difference in blood cortisol concentrations. A study in male Criollo goats yielded similar results (Ortiz de Montellano et al 2007).

Like cortisol, adrenal progesterone is also secreted by the adrenal glands in the Hypothalamic Pituitary Adrenal (HPA) axis stress response and is considered as an indicator of stress in cattle (Whitlock et al 2012). Etson et al (2004) reported that changes in blood progesterone concentrations may be a more sensitive indicator of pain and stress during EEJ, due to the greater effect epidural lidocaine had on reducing blood progesterone concentrations when compared with blood cortisol (Etson et al 2004). Falk et al (2001) also contend that blood progesterone is potentially a more sensitive indicator of pain or stress during EEJ because, unlike cortisol, transrectal massage alone increased blood progesterone concentrations significantly in bulls (Falk et al 2001).

Substance P is a neuropeptide involved in the pain, stress and anxiety response (Whitlock et al 2012). Only one study on 9 Angus bulls examined Substance P in conjunction with blood cortisol and progesterone and vocalisations during EEJ. Whilst vocalisations, blood cortisol and progesterone increased in response to EEJ, no significant increase in Substance P was recorded (Whitlock et al 2012).

Change in heart rate (HR) during EEJ has been evaluated as a potential pain indicator. The HR change associated with EEJ is most likely due to a combination of pain from the electrical stimulus and muscle contraction (Boussena et al 2013). Muscle exertion of any kind, including muscle contractions elicited during EEJ and normal sexual activity, can lead to an increase in HR, prompting some authors to conclude that HR changes may not be a reliable measure of pain during EEJ (Mosure et al 1998). This study compared HR in bulls with and without epidural lidocaine, HR was higher in bulls during EEJ that had not received a lidocaine epidural.

The Effects of Anaesthesia to on Pain Responses to EEJ

Almost all studies on the effects of anaesthesia and analgesia on pain during EEJ show some reduction in pain-related behavioural and physiological responses.

Intrarectal lidocaine reduced the sensation to the perirectal area causing a reduction in the number and strength of rectal smooth muscle contractions. Bulls treated with intrarectal lidocaine showed the same increase in HR during EEJ as bulls without intrarectal lidocaine, from which the authors concluded that pain associated with EEJ is more likely due to nerve over-stimulation rather than spasm of rectal smooth muscle (Mosure et al 1998).

Lidocaine epidural anaesthesia is the most researched method of pain reduction during EEJ and its use does not impact on penile protrusion or semen emission (Mosure et al 1998) or spermatogenesis (Pagliosa et al 2015). Epidural lidocaine was reported to reduce elevations of cortisol, progesterone and HR during EEJ in bulls, but the reductions were not significant (Falk et al 2001, Etson et al 2004). Furthermore, fewer bulls vocalised during EEJ with a lidocaine epidural, but the difference was not significant (Falk et al 2001). Etson et al (2004) reported that bulls exhibited little difference in behavioural responses across all treatments including lidocaine epidural, indicating that lidocaine epidural anaesthesia is only minimally effective in reducing pain and stress associated with EEJ.

Epidural xylazine was more effective in reducing the physiological pain response in bulls to EEJ, resulting in less elevation in HR (although not significantly) and less muscle exertion than both xylazine IV and lidocaine epidural and maybe more useful as an analgesic (Mosure et al 1998). This study reported that xylazine treated bulls, regardless of administration method frequently became recumbent in the race before or after EEJ and typical xylazine side effects were apparent. A recent study looked at 3 types of epidural treatments (plus a saline control) as pain reducing measure during EEJ in 8 bulls, habituated to the handling facilities, but from which semen had not previously been collected (Pagliosa et al 2015). Using a scoring system for evaluating pain, discomfort and ataxia in bulls, treatment with xylazine epidural or xylazine plus hyaluronidase epidural resulted in less pain and discomfort than treatment with lidocaine epidural. Xyalzine with hyalurondiase was the most effective treatment overall (Pagliosa et al 2015). None of the epidural treatments had any significant effect on HR during EEJ, although both xylazine treatments produced significantly lower HR when compared to the control group at 20 and 30 minutes after EEJ. None of the bulls lay down during the procedure, however some of the xylazine and xyalzine plus hyaluronidase group became recumbent temporarily after leaving the handling facilities post EEJ (Pagliosa et al 2015).

A number of drugs and techniques may ease the adverse effects of EEJ by reducing the intensity and duration of electrical stimulation required for EEJ. In a study on the use of oxytocin, a hormone that increases in association with ejaculation, it was shown that if given prior to EEJ, oxytocin helped to reduce the time to semen emission significantly. However oxytocin failed to have an effect on the number of EEJ stimuli, so it is not useful to alleviate pain during EEJ (Palmer et al 2004). Prostaglandin F2α is another hormone that is involved in the ejaculation process and improves smooth muscle contractility in males. Cloprostenol is a potent Prostaglandin F2α analogue and its use had no effect on time to semen emission or on the number of EEJ stimuli required to produce ejaculation (Palmer et al 2004).

<u>Alternative Techniques to EEJ</u>

Techniques such as transrectal massage in advance of EEJ had no effect on time to semen emission or on the number of electrical stimuli required for ejaculation (Palmer 2004). However, rectal massage targeted specifically at the ampullae (specific rectal massage) reduced the time to semen emission and also the number of stimuli required and can potentially be used as a method of pain reduction during EEJ (Palmer et al 2004). Palmer et al (2005b) concluded that specific rectal massage of the ampullae can be used as a method of semen collection in 80% of bulls unaccustomed to handling and 95% of yearling bulls accustomed to handling. Sperm morphology was not affected by collection method. However it should be noted that sperm characteristics such as motility and number of live sperm were lower in sampled collected by specific rectal massage and could compound results interpretation in breeding soundness examination (Palmer et al 2005b).

Equipment & Operator factors affecting pain response during EEJ

The influence of probe type used during EEJ, with or without epidural lidcocaine anaesthesia, on blood cortisol and progesterone concentrations was investigated by Etson et al (2004) in ten 16 month old bulls. The bulls were habituated to the race and semen collection area over a 1 week period in advance of the experiment, minimising the impact of handling/restraint on blood cortisol and progesterone levels. The frequency, duration and voltage of electrical stimuli were applied similarly across all bulls. Serum cortisol and progesterone became elevated immediately following EEJ irrespective of probe type or pretreatment with a lidocaine epidural, suggesting that the use of segmented over non segmented probes was not effective in reducing pain during EEJ (Etson et al 2004).

Stimulus duration, rest intervals and voltage increments can also influence the level of pain response observed (Stafford 1995). When compared with a stimulus rest interval of 5 seconds, a 10 second interval greatly reduced the physical response of rams to EEJ (Stafford 1995). Palmer (2005a), recommends close monitoring of bull's behaviour during EEJ whereby once the bull starts to react, the stimulus is removed for 0.5-1 seconds. With each successive stimulation of 1-2 seconds, the voltage is steadily increased.

The correct placement and orientation of the probe is important to minimise the pain response to EEJ (Stafford 1995). Differences between studies on pain indicators during EEJ may stem from variations in operator technique. Perhaps once operator technique is standardized and improved, a reduction in pain responses may be observed (Palmer 2005a).

Regulatory Frameworks in Other EU Member States

A survey of national contact points in five countries was conducted, in order to review approaches taken in other jurisdictions (Table 2). The procedure is prohibited in Denmark and the Netherlands (except for zoo animals, where EEJ must be conducted with full anaesthetic and under veterinary supervision). The UK regulates the practice, by requiring veterinary supervision. In Germany, the respondent indicated that it is not a routine practice, and mainly performed in zoo animals.

Discussion

Relatively few scientific studies have been conducted on EEJ. Differences in experimental design and small sample sizes (Table 1) may help to explain variation in the results. However, despite the limitations of the published studies, there is evidence to indicate that EEJ is a painful procedure, based on behavioural data, in particular, vocalisations, and physiological indicators, blood cortisol and heart rate. In addition, epidural anaesthetic was shown to reduce pain responses to EEJ (Falk et al 2001, Etson et al 2004).

A number of contributory factors to the pain response associated with EEJ have been identified such as the level of electrical stimulation (Falk et al 2001; Fumagilli et al 2012), operator technique (Grandin 2010; Palmer 2005a). Studies have recommended that the voltage of the EEJ probes should be under the control of the operator at all times. EEJ should start with a low level setting and increased until ejaculation occurs. Modern EEJ equipment has pre-programmed current patterns considered appropriate for specific-species, which deliver a series of stimuli of increasing intensity until ejaculation occurs (Stafford 1995).

Many of the studies on EEJ refer to animal welfare concerns over the use of this procedure. Between 1991-1995 the EU banned the importation of frozen semen collected by EEJ because the procedure was considered inhumane (Mosure et al 1998, Falk et al 2001). Furthermore semen collection by EEJ is currently banned in several European countries (Palmer 2005a, Mosure et al 1998, Falk et al 2001) such as the Netherlands and Denmark and animal welfare campaigners in Europe have been calling for the procedure to be banned (Stafford 1995). In the UK, EEJ has to be conducted under the supervision of a veterinary surgeon.

Pain reduction as a result of lidocaine epidural during EEJ varied with study (Mosure et al 1998; Etson et al 2004, Falk et al 2001), leaving authors questioning the harm-benefit of using epidural lidocaine (Palmer 2005a, Etson et al 2004). Xylazine resulted in reduction in heart rate elevation and muscle exertion of bulls undergoing EEJ (Mosure et al 1998) and animals appeared calmer (Pagliosa et al 2015). However Stafford (1995) reported adverse effects with the use of xylazine such as increased salivation, bellowing and increased respiratory effort. Furthermore, Xylazine can take 20-30 minutes before the analgesic property takes effect. The addition of hyaluronidase (enzyme) to the xylazine epidural accelerates the onset of the analgesic effect of the local block by improving the spread of the locally applied (epidural) xylazine (Pagliosa et al 2015).

Various administration routes and their efficacy have been investigated, however, it should be noted that although xylazine is licensed for use in cattle in Ireland, it is only licensed for use via the intravenous or intramuscular routes (6) and its use as an epidural agent would involve using the product off-label. In conclusion, research on anaesthesia and analgesia during EEJ may help to lower pain and discomfort during EEJ. However the risks associated with this general anaesthetic protocol are not recommended for ruminants.

Acknowledgements

The Scientfic Advisory Committee on Animal Health and Welfare would like to thank Bernadette Doyle, Liam Barry and Padraig Jennings for their assistance with the literature review.

References

- **Websites**

1. Semen Collection. Available from http://www.vivo.colostate.edu/hbooks/pathphys/reprod/semeneval/collection.html[accessed 13th September 2015].
2. Electro-ejaculation. Available from https://en.wikipedia.org/wiki/Electroejaculation [accessed 13th September 2015].
3. Canadian Veterinary Medical Association (CVMA) position statement on Electro-ejaculation. Available from http://www.canadianveterinarians.net/documents/electroejaculation-of-ruminants[accessed 13th September 2015].
4. Overview of Breeding Soundness Exam of the Male. Available from www.merckvetmanual.com [accessed 13th September 2015].
5. The Health Products Regulatory Authority Veterinary Medicines listing. Available from www.hpra.ie [accessed 24th September 2015].
6. The Veterinary Surgeons Act 1966 (UK). Available from http://www.legislation.gov.uk/ukpga/1966/36 T0DQI4M08R5 [accessed 26th September]
7. Semen Collection from Stallions. Available from http://www.vivo.colostate.edu/hbooks/pathphys/reprod/semeneval/stallion.html [accessed 27th September 2015].
8. Semen Collection by Electroejaculation. Available from Xcell breeding, https://www.facebook.com/XcellBreeding/posts/345829092254990 [Accessed 27th September 2015].

- **Publications:**

Barth A.D., Bowman, P.A. 1994. The sequential appearance of sperm abnormalities after insulation or dexamethasone treatment in bulls. Canadian Veterinary Journal, 35, 93-102.

Bath G.F. 1998 Management of pain in production animals. Applied Animal and Behavioural Science, 59, 147-156.

Brindley, G.S. 1981.Electroejaculation: its technique, neurological implications and uses. Journal of Neurology, Neurosurgery and Psychiatry, 44, 9-18.

Boussena, S., Bouaziz, O., Dehim, M.L., Hireche, S., Aimeur, R., Kasouia, R. 2013. The effects of electroejaculation on some physiological parameters (rectal temperature, respiratory and cardiac rates) in Ouled Djellal breed. Slovakian Journal of Animal Science, 46, 1, 16-21.

Cary J.A., Madill, S., Farnsworth, K., Hayna, J.T., Duoss, L., Fahning M.L. 2004. A comparison of electroejaculation and epididymal sperm collection techniques in stallions. Canadian Veterinary Journal 2004, 45(1), 35–41.

Damian J.P., Ungerfield, R. 2011. The Stress response of frequently electroejaculated rams to electroejaculation: hormonal, physiological, biochemical, haematological and behavioural parameters. Reproduction in Domestic Animals, 46, 646-650.

Etson C.J., Waldner C.L., Barth A.D. 2004. Evaluation of a segmented rectal probe and epidural anesthesia for electro-ejaculation of bulls. Canadian Veterinary Journal, 45 (3), 235-240.

Falk AJ, Waldner CL, Cotter BS, Gudmundson J, Barth AD. 2001. Effects of epidural lidocaine anesthesia on bulls during electroejaculation. Canadian Veterinary Journal, 42 (2), 116-120.

Fumagalli, F., Villagran,M., Damián, J.P., Ungerfeld, R. 2012. Physiological and biochemical parameters in response to electroejaculation in adult and yearling anesthetized pampas deer (Ozotoceros bezoarticus) males. Reproduction in Domestic Animals, 47, 308–312.

Grandin, T. 1998. The feasibility of using vocalisation scoring as an indicator of poor welfare during cattle slaughter. Applied Animal Behaviour Science, 56, 121-128.

Grandin, T. ed. 2010. Improving Animal Welfare: A Practical Approach. 2nd ed. Colorado State University, CABI, p.11.

Leibo, S.P., Songsasen, N. 2002. Cryopreservation of gametes and embryos of non-domestic species. Theriogenology, 57, 1, 303-326.

Moberg GP. 2000. Biological Response to Stress: Implications for Animal Welfare. In GP Moberg and JA Mench (Eds) The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. CABI Publishing. Pp 1-21.

Molony, V., Kent, J.E. 1997. Assessment of acute pain in farm animals using behavioural and physiological measurements. Journal of Animal Science, 75, 266-272.

Mosure W.L., Meyer R.A., Gudmundson, J., Barth A.D. 1998. Evaluation of possible methods to reduce pain associated with electro-ejaculation in bulls. Canadian Veterinary Journal, 39 (8), 504-506.

Orihuela, A., Aguirre, V., Hernandez, C., Flores-Perez, I., and Vazquez, R. 2009a. Breaking down the Effect of Electro-Ejaculation on the Serum Cortisol Response, Heart and Respiratory Rates in Hair Sheep (Ovis aries). Journal of Animal and Veterinary Advances, 8 (10), 1968-1972

Orihuela, A., Aguirre, V., Hernandez, C., Flores-Perez, I., and Vazquez, R. 2009b. Effect of anaesthesia on welfare aspects of Hair Sheep during electroejaculation. Journal of Animal and Veterinary Advances, 8 (2), 305-308.

Ortiz-de-Montellano, M., Galindo-Maldonado, F., Cavazos-Arizpe, E.O., Aquayo-Arceo, A.M., Torres Acosta, J.F.J., Orihuela, A. 2007. Effect of electroejaculation on the serum cortisol response of Criollo goats. Small Ruminant Research, 69, 228-331.

Pagliosa, R.C., DeRossi, R., Costa, D.S., Faria, F.J. 2015. Efficacy of caudal epidural of lidocaine, xylazine and xylazine plus hyaluronidase in reducing discomfort produced by electroejaculation in bulls. The Journal of Veterinary Medical Science [Advance Publication 22nd June 2015].

Palmer C.W. 2005a. Welfare aspects of theriogenology: investigating alternatives to electro-ejaculation of bulls. Theriogenology, 64 (3), 469-479.

Palmer, C.W., Amundson, S.D., Brito, L.F., Waldner, C.L., Barth, A.D. 2004. Use of oxytocin and cloprostenol to facilitate semen collection by electro-ejaculation or transrectal massage in bulls. Animal Reproduction Science, 80 (3-4), 213-223.

Palmer, CW., Brito, L.F., Arteaga, A.A., Soderquist, L., Persson, Y., Barth, A.D. 2005b. Comparison of electroejaculation and transrectal massage for semen collection in range and yearling feedlot beef bulls. Animal Reproduction Science, 87 (1-2), 25-31.

Rushen, J. 1986a. Aversion of sheep to electroimmobilisation and physical restraint [Abstract]. Applied Animal Behaviour Science, 15, 315-324.

Rushen J. 1986b. The validity of behavioural measures of aversion: a review [Abstract]. Applied Animal Behavioural Science, 16, 309-323.

Rushen, J.1996. Using aversion learning techniques to assess the mental state, suffering, and welfare of farm animals. Journal of Animal Science, 74 (8), 1990-1995.

Shipley, C.F. 1999. Breeding soundness examination of the boar. Swine Health Production, 7 (3), 117-120.

Short, C.E. Fundamentals of pain perception in animals. 1998. Applied Animal Behaviour, 59, 125-133.

Stafford, K. J. 1995. Electro-ejaculation: a welfare issue. Surveillance, 22, 15-17.

Szechtman, H., Lambrou, P.J., Caggiula, A.R.,Redgate, E.S. 1974. Plasma corticosterone levels during sexual behavior in male rats [Abstract]. Hormones and Behaviour, 5, 191-200.

Vineula-Fernandez, I., Jones, E., Welsh, E.M., Fleetwood-Walker, S.M. 2007. Pain mechanisms and their implications for the management of pain in farm and companion animals.The Veterinary Journal, 172, 227-239.

Weary, D.M., Lee, N., Flower, F.C., Fraser, D. 2006. Identifying and preventing pain in animals. Applied Animal Behaviour Science, 100, 64-76.

Whitlock B.K., Coffman E.A., Coetzee J.F., Daniel J.A. 2012. Electroejaculation increased vocalisation and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology, 78, 737-746.

Watts, J.M., Stookey, J.M. 1999. Effects of restraint and branding on rates and acoustic parameters of vocalisation in beef cattle. Applied Animal Behaviour Science, 62, 125-135.

Table 1. Summary of experimental studies investigating the effects of EEJ on pain responses in bulls

	Pagliosa et al, 2015	**Whitlock et al 2012**	**Etson et al 2004**	**Falk et al 2001**	**Mosure et al 1998**
Objectives	To evaluate the effectiveness of caudal epidural lidocaine, xylazine and xylazine plus hyaluronidase for reducing discomfort due to EEJ	To compare Substance P, vocalisations, cortisol and progesterone in bulls following EEJ	To determine effects of probe type (segmented electrodes vs nonsegmented) and caudal epidural anesthesia with lidocaine on pain responses to EEJ in bulls	To determine whether caudal epidural lidocaine reduces pain of EEJ in bulls	To evaluate possible methods of reducing pain due to EEJ by using heart rate change as an objective measure of pain.
Number & type of animals	8 x 30 month old Nellore bulls 405–448 kg, mean 425 kg Range bulls No previous experience of EEJ	9 x 14 month old Angus bulls 501.9 +/- 14.3 kg	10 x 16 month old Hereford bulls	Expt 1 - 5 x 2 year old Hereford bulls Previous experience of EEJ Expt 2 – 62 yearling bulls; 6 beef breeds No previous experience of EEJ	3 year old Hereford 2 year old Simmental 2 year old South Devon Previous experience of EEJ
Pretreatment	Habituated to the chute	Habituated to the chute	Habituated to the chute	Habituated to the chute	Habituated to the chute
Probe type	Ventral segmented probe - diameter of 75 mm (Duboi, Campo Grande, Brazil).	Ventral nonsegmented probe (3 electrodes) diameter 90 mm length 360 mm; A Lane Pulsator IV	Ventral nonsegmented probe (3 electrodes) 75 mm in diameter (Lane Manufacturing, Denver, Colorado, USA). Ventral segmented probe 75 mm in diameter with 3 short caudal electrodes, 3 short middle electrodes, and 2 short cranial electrodes (Kane Veterinary Supplies, Saskatoon, Saskatchewan).	Ventral probe 75mm diameter	Ventral probe 60mm diameter
Pattern of electrical stimulation	A standardised sequence of electric pulses (60 Hz) was administered: 30 shortwaves between 100 and 300 mA (lasting 2–3 sec with 0.5 sec	Sine wave pulse @ 15 Hz for one preprogrammed Cycle: 214 s period of gradual/incremental increases from 0 to 13 V and a maximum current of	Identical sequence of electrical pulses of increasing intensity for each treatment until 27 stimuli had been administered. Voltage across electrodes was	Preprogrammed sequence using Pulsator IV (Lane manufacturing, Denver, Colorado, USA)	Preprogrammed 4 s cycles (2s stimulation, 2 s rest periods); 3 stimulations at each V increment; manual 10 increments of voltage. Each bull received same max.

	intervals) followed by 2 long waves of up to 500 mA (lasting 3–5 sec).	900mA, with successive stimulation V intensity was steadily increased and held for 1 s, followed by 1 s of rest	measured & EEJ settings were adjusted to provide a similar output between probes. When the segmented probe was being used, the caudal 3 segments were active for the first 15 stimuli and the middle 3 segments for the last 12 stimuli		stimulation whether or not ejaculation occurred.
Treatments	saline solution (control), 2% lidocaine, 2% xylazine or 2% xylazine plus hyaluronidase injected into the first intercoccygeal (Co1–Co2) epidural space in randomized order	EEJ, insertion of a rectal probe without electrical stimulation (probed), or no treatment (control). Latin square design – each bull received each treatment	5 treatments, one/day was administered to 5 bulls in wk 1 & the remaining 5 bulls in wk 2. Day (D) 1: restraint only control; D 2: lidocaine epidural + EEJ (segmented probe); D 3: Lidocaine epidural + EEJ (conv probe); D 4: EEJ (segmented Probe); D 5: EEJ (conv probe)	Expt 1. 5 treatments: 1. control (restraint only) 2. transrectal massage for 2 min 3. saline epidural 4. lidocaine epidural 5. transrectal massage + EEJ Expt 2. EEJ (incl transrectal massage), with and without lidocaine epidural	6 treatments: transrectal massage of the vesicular and ampullary-prostate-urethral region for 3 min; conventional EEJ; intrarectal topical admin of lidocaine 5 min before EEJ;xylazie epidural 25 min before EEJ; IV xylazine sedation 5 min before EEJ
Pain Indicators	Heart rate, respiratory rate, mean arterial pressure, analgesia, animal behavior (scoring system: pain, discomfort and ataxia; vocalisations, restlessness)	Vocalisation, plasma cortisol, progesterone, and substance P immunoreactivity	Behaviour (vocalization, struggling, recumbency), serum cortisol and progesterone concentrations	Expt 1: Cortisol and progesterone Expt 2. Behaviour: vocalizations (0-4); recumbency and struggling	Heart rate
Key findings	Less discomfort with xylazine or xylazine + hyaluronidase than with lidocaine, as indicated by animal behaviour. Changes in heart rate, respiratory rate and arterial pressure were within acceptable limits.	Greater vocalisations in EEJ group vocalised (5 of 9 bulls; 55.6%) compared to controls (0 of 9 bulls; 0%). Mean plasma cortisol and progesterone concentration following EEJ were higher (P_0.05) than other treatments through the 45	Cortisol and progesterone increased after EEJ compared to restraint alone. Epidural reduced the increases in progesterone at 5 and 20 minutes after EEJ. No difference in cortisol or progesterone after EEJ between conventional and	Cortisol was significantly elevated above pretreatment 25 min after lidocaine epidural (14.7nmol/L) & conventional EEJ (22nmol/L), cortisol decreased in conv EEJ by 45 min, but remained elevated	Heart rate greater (P=0.08) for EEJ and EEJ with topical intrarectal lidocaine than for transrectal massage and EEJ with lidocaine epidural

		min sample. No significant treatment difference (P =0.63) in mean plasma substance P	segmented probe. Subjective scoring showed no differences among EEJ methods.	for lidocaine epidural. PG exceeded pretreatment levels for lidocaine EEJ & massage 5 and 25 min after treatment. PG elevated above pretreatment levels for conv EEJ, massage and control 45 min post treatment. Inconsistent results for behavioural measures	

Table 2 Summary of survey responses by national contact points in 5 EU member states regarding regulation on electro-ejaculation

	Belgium	**UK**	**Denmark**	**Netherlands**	**Germany**
Regulations prohibiting EEJ	No	No	Yes Prohibited in bovine and fur animals	Yes Except for zoo animals	No (Only performed in zoo animals)
Registered operators	No	Has to be conducted under veterinary supervision	No	Has to be conducted under veterinary supervision	No
Codes of practice	No	No	N/A	N/A	No information
Requirements on EEJ equipment	No	No	N/A	No information	N/A
Is anaesthesia required	N/A	Sedation may be required	No information	Yes (for zoo animals)	Yes (for zoo animals)

Table 3 Mean number of vocalizations per bull in response to restraint in handling facilities (control) or restraint plus EEJ using either a high or low voltage apparatus

	Control - Restraint only	High voltage EEJ machine	Low voltage EEJ machine
Mean number of vocalisations per bull	0.15 + 0.1	8.9 + 1.1	3.9 + 1.0

Source: Grandin (2010) Improving Animal Welfare: A Practical Approach pp11