A refresher on sperm morphology

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Introduction

The analysis and reporting of the spermiogram under current ACV BBSE BULLCHECK® guidelines is unique in the world as it uses central laboratories to provide unbiased expert analysis of sperm morphology under a standardised national assessment system. This assessment system (Perry et al., 2002; Entwistle and Fordyce, 2003; McAuliffe et al., 2010), determines the threshold levels of sperm abnormalities based upon published research into the effects of each abnormality upon fertility. The importance of this scheme within the standardised bull breeding soundness examination (Fordyce et al., 2006) is accepted as the gold standard for sperm morphology examination and widely cited.

A major hurdle to the incorporation of morphology into Breedplan, and uptake by the cattle industry in general, is the observed environmental effects upon morphology figures. These include climate (Felton-Taylor et al., 2018) and particularly nutrition either; in utero (Copping et al., 2017), pre weaning (Brito et al., 2007c; Callaghan and Perry, 2010) or pre sale (Callaghan et al., 2016). It is clear however, that a few sperm morphology abnormalities are heritable and furthermore a strong trend exists for morphology to be significantly poorer in some breeds than others (Hoflack et al., 2006; Felton-Taylor et al., 2018).

A further important hurdle in the wider acceptance of sperm morphology as part of the BULLCHECK® BBSE is the lack of standardisation of central laboratories, particularly since 2011. Measures to address these issues will be discussed in this review.

Discussion

This ACV BBSE BULLCHECK® scheme differs from that in use for example in the US, Canada and the UK where morphology examination is usually completed crush side using vital stains such as nigrosin eosin which enable assessment of morphology under bright field microscopy. This method has been shown to be inaccurate in its assessment of morphological abnormalities, particularly head abnormalities, in many studies (Sekoni et al., 1981; Sprecher and Coe, 1996; Al-Makhzoomi et al., 2008; Freneau et al., 2010; Palmer et al., 2013) when compared to the assessment of wet mounts under DIC (Differential Interference Contrast) or phase contrast microscopy usually completed in a laboratory. The pros and cons of crush side vital staining compared with DIC at central laboratories will be covered in the session.

The considered professional gold standard for both the assessment of bovine and equine sperm morphology is DIC microscopy at x1000 magnification. This is the ACV recommended standard for Australian laboratories. Samples are sent to the central laboratories in buffered formal saline, which enables high quality wet preparations to be examined by the morphologist. Even with this level of microscope it is still necessary to focus up and down on each sperm to accurately assess abnormalities at the limit of resolution. This, however, is less the case than is necessary with Phase contrast microscopy. In recent years the advance of the DIC microscope has enabled even difficult to detect abnormalities of the DNA e.g. pale centres, to be viewed without the aid of Feulgen staining, although the latter is instigated as a base check.

Fertile bulls have a spermiogram, which contains <30% abnormal sperm (Wiltbank and Parish, 1986; Fitzpatrick et al., 2002; Holroyd et al., 2002). Immediately following the ground breaking work by Holroyd’s Australian Bullpower team, the ACV instigated this threshold level and, importantly, developed individual thresholds for each abnormality (Perry, 2002) following a single standard (Entwistle and Fordyce, 2003; Fordyce et al., 2006). Sperm cells were classified into the 8 standardised categories indicated in this publication. Thresholds for each sperm abnormality (category) vary and are based upon the currently known effect upon fertility. In conducting the spermiogram, each defect on each abnormal sperm is recorded; that is, more than one defect may be recorded per sperm. This differs from the UK where no threshold levels per abnormality exist only the criteria that >70% normal sperm should be observed. In Canada there is an overarching threshold of no individual head abnormality being present at >20% in addition to >70% normal sperm. Under the Society for Theriogenology (SFT) system in the US, a similar overarching threshold exists but at >30% for any individual abnormality. It should be noted however that this SFT system is under review and may more closely resemble the Canadian system (Kastelic, Palmer pers com) in future.

A minimum of 100 sperm are counted per spermiogram under the ACV system (McAuliffe et al., 2010), which is similar in the UK, US and Canada. This however, is increased to 200 in BULLCHECK® in the case of borderline (62-77) counts based on published data (Kuster et al., 2004). This standardised increase in counts for borderline cases was instigated in 2016 following a meeting of morphologists interested in improving standardisation across laboratories (Perry, 2017).

The 8 categories in the ACV BBSE BULLCHECK® scheme with tolerance levels are, in order; normal sperm- which includes abnormalities observed but that are considered not to effect conception rates, proximal droplets (20%), midpiece abnormalities (30%), loose heads and principle piece (tail) abnormalities (30%), pyriform heads (20%), knobbed acrosomes (30%), vacuoles and teratoids (including abnormalities of DNA condensation) (30%), swollen acrosomes (including those sperm with lost acrosomes) (30%)(Fordyce et al., 2006). These 8 categories were originally determined by time and the functions of the 8 stage manual counter. The advent of the keyboard counter system has however allowed further subcategories to be readily included without increased time input. These may be observed on the advanced format sheet if the veterinarian wishes further information. This is not available on the UK, US BBSE sheets. These advanced input sheets have in the past year allowed sub categorisation of, for example; flat acrosomes, pale centres, abaxial tails, and the 3 categories of vacuolation(Perry, 2017).

In 2002 the ACV (Entwistle and Fordyce, 2003) adopted the terminology put forward by Evenson et al., (1999) who considered that semen may contain both compensable and uncompensable sperm traits. Understanding the compensable uncompensable concept is simple if we consider that the female tract (Mitchell et al., 1985) and finally the vestments of the ovum act as a filtration system for the sperm population. They act as barriers to the progress of sperm such that only the fittest arrive at the ovum (Saacke et al., 1998). Compensable traits preclude affected spermatozoa from fertilizing the ovum, i.e. the abnormality does not allow them either to reach the ova or attach to the ova. A compensable abnormality, therefore, is one that can be compensated for by increasing the number of spermatozoa in the ejaculate; that is the fertility of the bull will increase with increasing numbers of spermatozoa. These include traits, which cause, for example, abnormal or nil motility (these are filtered out in the female tract), and abnormal head shape (filtered out crossing the zona as these interfere with hyperactive motility required at this juncture). The threshold for such abnormalities is set at 30% (Johnson, 1997; Perry, 2002; McAuliffe et al., 2010). Increasing numbers of spermatozoa, however, cannot compensate for uncompensable traits. Sperm with these traits are able to reach the ovum and initiate fertilization (thereby blocking polyspermy) and/or embryo development but that development is unsustainable. The cow therefore returns to oestrus. Such traits include, nuclear vacuoles and pyriform heads. They tend to be the subtler more difficult to detect abnormalities yet cause the biggest decrease in conception rates. The suggested threshold of such abnormalities is therefore 20% (Perry, 2002; McAuliffe et al., 2010). In general it could be said that sperm with abnormalities that do not allow them to reach the ova or attach are considered compensable traits. Those abnormalities, which allow the sperm to fertilize the ova but result in early embryonic death or abnormal development, are considered uncompensable.

By conducting spermatozoon morphology differentials in this manner we can achieve a standard and accurate prognosis of the bulls performance. A summary of the current understanding of the severity and consequence of the various spermatozoon abnormalities is contained in the supplementary data. It is not all conclusive and contributions from further research will occur over time.

Pathogenesis of Abnormal Sperm Production:

(a) Stress in the form of pain, hunger or cold may affect both spermatogenesis and epididymal function as high levels of circulating cortisol suppress the requisite testosterone concentrations to these organs. This is neatly shown in the publication by Callaghan (Callaghan et al., 2016) where a single acidotic event was followed sequentially by elevated cortisol, reduced FSH and testosterone consequent with increased sperm abnormalities in the subsequent weeks. Similarly stress associated with transport and relocation (Perry, 2017) or dexamethasone (Barth and Bowman, 1994) can affect the spermiogram

(b) Heat whether due to obesity, scrotal abnormality, climate or fever has been observed to increase sperm abnormalities in the bull ejaculate(Barth, 2013b). Mechanisms that maintain testis homeothermy include the cremaster and dartos muscles and the pampiniform plexus. This latter consists of coiled veins, which surround and cool the incoming testicular blood. A distinct scrotal neck is necessary which may be absent in the obese animal. The testis are particularly susceptible to heat as testicular function occurs in a marginally hypoxic environment where an increase in temperature may increase in metabolic rate, but there is no corresponding increase in blood flow. Tissues are therefore susceptible to hypoxia(Barth and Bowman, 1994). A recent study,(Felton-Taylor et al., 2018),found the number of bulls passing the sperm morphology test at 70% were reduced in Far Northern Australia however, climatic region had less effect than breed upon the numbers of bulls with poor morphology.

(c) Season affects sperm morphology in the bull although not as distinctly as the ram where photoperiod clearly alters sperm output(Hötzel et al., 2003). The effect of season in the bull is considered to be influenced by the prevailing nutrition and temperature (Entwistle and Fordyce, 2003). A recent report (Felton-Taylor et al., 2018) shows that, although total number of bulls passing the sperm morphology test was similar in all seasons in Australia, there was an elevation in bulls failing the knobbed acrosome and vacuole thresholds in the summer months.

(d) Heredity – some sperm defects may be inherited, for example, the knobbed acrosome, which is inherited by an autosomal recessive gene(Hancock, 1953; Barth, 1986)and the Dag defect (Blom, 1966).

(e) Breed- the spermiogram of bulls of certain breeds have been reported to be higher in morphological abnormalities than others, for example the Belgian blue compared to the Friesian (Hoflack et al., 2006) and *Bos indicus* breed bulls compared to *Bos taurus* breeds (Felton-Taylor et al., 2018). A Canadian study (Menon et al., 2011), however, showed no effect of breed on the spermiogram between *Bos taurus* breeds

(f) Pubertal and peri pubertal bulls display a disturbed spermiogram until maturity is reached(Perry et al., 1991; Brito et al., 2007a). Particularly high levels of proximal droplets occur during this period and these may vary between ejaculates collected on the same day (Gardner, B, Perry V pers.com,)(Copping et al., 2017).

(g) Toxicity-a common concern in Australia where cottonseed is regularly fed to breeding animals is the possible effect of gossypol upon sperm morphology. Abnormalities have been shown to increase in some studies (Chenoweth et al., 2000) but not in others (Cusack and Perry, 1995). The dietary supply of metallic cations (e.g. calcium, iron) is thought to cause this differential effect as these bind gossypol in the rumen (Cusack and Perry, 1995) and may be present in, for example, the mineral content of bore water or when lime is added to the diet.

(h) Nutritional deficiencies of major nutrients, whether prenatal (Copping et al., 2017), pre weaning(Brito et al., 2007b; Barth et al., 2008; Callaghan and Perry, 2010) or pre sale(Perry, 2017) have been shown to affect maturation of the spermiogram. During adulthood nutritional restriction and/or dietary change may have deleterious effects particularly in bulls predisposed to developing certain sperm abnormalities such as nuclear vacuoles (Callaghan et al., 2016)

Standardisation

The lack of standardisation between laboratories used by veterinarians has increased since 2011 when the ACV accreditation system ceased. An anecdotal example of such variation this year showed one laboratory stating an abnormality at >40% whereas three other laboratories found <10% of the same abnormality over 4 shared ejaculates. This situation is clearly frustrating for the veterinarian concerned and costly to the cattle industry. The failure of the profession and the cattle industry to reinstate a proficiency system is a major flaw to the improvement of bull fertility in Australia. There is the potential for monetary gain in the passing of bulls on their sperm morphology test. This has been also noted in Canada where in order to maintain a license to practice it is a requirement of their annual inspection that semen smears and records are kept for potential inspection. Fines are applied and names publicised in the WCABP association newsletter of transgressions.

Under the ACV system it was a requirement that samples were kept for 2 years and that these samples were made available to accredited examiners where a disagreement arose. There is currently no such facility for checking of past work and the ACV has dropped the requirement that samples are kept. It considers that sample identification per bull could not be verified. The majority of laboratories however, do keep samples for at least 2 years.

The University of Queensland has held meetings of sperm morphologists interested in standardising their reporting of sperm abnormalities. These were held in 2014, 2015, 2016 and samples distributed from 3 laboratories for each morphologist to compare with one another and with a group median count determined by a UQ statistician. Unfortunately these meetings have not continued despite enthusiastic participation from attendees.

Conclusion

It is established that the level of normal sperm in the ejaculate of the fertile bull should be >70%(Wiltbank and Parish, 1986; Fitzpatrick et al., 2002; Holroyd et al., 2002). However, this figure should be interpreted by the laboratory according to the type of abnormalities contained within the sample(Fordyce et al., 2006; McAuliffe et al., 2010; Barth, 2013b). The laboratory sending out the results of the analysis should have the ability to give a prognosis to the client based upon their knowledge of spermatogenesis together with information such as that provided by a full bull breeding soundness examination(Entwistle and Fordyce, 2003).

This ACV model has enabled increased accuracy of prognosis for practitioners and is well regarded both in Australia and overseas (Irons et al., 2007; Penny, 2010). The lack of standardization among these central laboratories, however, threatens the use of morphology as a reliable tool for practitioners as well as its standing within the cattle industry. Progression on the reinstatement of a standardization of Australian laboratories is required.

Supplementary Data: Sperm abnormalities and accepted ACV thresholds.

<u>Proximal Droplets (PD):</u> These are normally observed in the pubertal bull with incidence decreasing with age (Amann et al., 2000; Copping et al., 2017). In the mature bull they indicate abnormal spermiogenesis (and/or epididymal function). They were observed 7-10 days following a temperature or stress event (Barth and Bowman, 1994) and 15 days following ruminal acidosis (Callaghan et al., 2016). The prognosis depends upon the type of abnormalities associated with the proximal droplets. Counts of 10-15% proximal droplets (Barth et al., 2008)have been associated with decreased fertility. This trait is considered uncompensable as the spermatozoa fail to bind to the ova and furthermore that sperm associated with high numbers of proximal droplet sperm have impaired ability to bind with the ova (Thundathil et al.). Amann et al., (2000) also found that in bulls with >30% proximal droplets that the associated apparently normal spermatozoa displayed immaturity and reduced ability to fertilize ova. This defect has a threshold of 20% as, both following AI and natural service, studies show proximal droplets are associated with poor pregnancy rates.

Distal Droplets. Unlike the boar there are no reports of distal droplets being associated with infertility in the bull. Spermatozoa with distal droplets will lose the droplet if left in a water bath for 15-30 minutes or if gently agitated. The number of sperm within the ejaculate with distal droplets, also vary widely between sequential ejaculates (McAuliffe et al., 2010). Case studies using bulls with high numbers of distal droplets in natural service achieve normal pregnancy rates (Barth, 2013a).For this reason distal droplets in isolation are not generally considered to be a defect by the author or by other researchers(Johnson, 1997; Barth, 2013a) and are placed in the normal category.

The presence of a cytoplasmic droplet whether in the proximal or distal position may be an indication that the sperm has not acquired essential binding proteins from the surrounding seminal fluid. These binding proteins are essential for the sperm to bind to the zona pellucida. For this reason it is important that massage of the ampullae and seminal vesicles is sufficient to illicit a quantity of seminal fluid during the collection process(McAuliffe et al., 2010).

Pyriform Heads (PY): Narrow in the postacrosomal region. Young bulls up to 2 years old and in good condition display a greater likelihood of recovery from this condition than do older bulls. This condition is particularly seen in young over fat bulls. It should be noted, that there is variation in this abnormality, in a series of experiments Barth (1992) showed that fertility was related to the severity of pyriformity of the head. A moderate degree of pyriformity, in the absence of other signs of disturbed spermatogenesis, is not detrimental to fertility. However, extreme tapering in the postacrosomal region results in significant reductions in fertility. Pyriformity is considered only partially compensable(Thundathil et al.) As in this study some pyriform sperm were able to fertilize oocytes but these had a reduced ability to cleave. The threshold of not more than 20% is therefore applied to this abnormality

Knobbed Acrosomes (KA): This is an abnormal pathogenesis that may be heritable or may be due to disturbances in the testis. Two forms occur; beaded and indented, however a third form; flattened, is often observed that is considered a subcategory of the indented form (Thundathil et al., 2000; Meyer and Barth, 2001). The beaded form is considered inherited by an autosomal recessive gene (Hancock, 1953; Barth, 1986). The indented form is described as an enlargement of the apical ridge that then folds back on the apex of the sperm head and is much more common than the beaded form. The beaded form is often associated with sterility and usually occurs as a high percentage of the ejaculate. Indented or flattened acrosomes vary in their effect upon fertility. In non-competitive matings such bulls may achieve near normal fertility however this may reflect that normal sperm coexisting with these sperm are in sufficient numbers to achieve conception as sperm with the flattened or indented form were unable to penetrate the zona pellucida (Thundathil et al., 2000). This abnormality is therefore considered a compensable defect and is given a 30% threshold.

This is supported by work by Andersson et al., (1988) who found that when present in less than 25% of sperm there was no decrease in fertility. In bulls with a high percentage of this abnormality (>80%) the indented acrosome defect may not be compensable as in such sperm did not bind to the zona pellucida and other sperm present in the ejaculate that appeared normal could bind to the zona the resulting zygotes had a reduced ability to cleave (Thundathil et al., 2001).

Swollen Acrosomes (SA): These are given a separate category as swelling and sloughing of the acrosome is a normal progression during sperm aging. The problem can be associated with "rusty load/ accumulated sperm" and the precautions suggested (McAuliffe et al., 2010) should be taken when collecting the semen. Ageing of the sperm causes the acrosome to undergo a similar reaction to capacitation resulting in the lifting of the acrosome, and clearly the sperm will not attach to the oocyte. Swollen acrosomes are often seen in conjunction with other head abnormalities such as knobbed acrosomes. This is because this abnormality causes premature initiation of the acrosome reaction (Thundathil et al., 2001). The swollen acrosome in these cases may hide the knobbed defect in initial observations. In these cases spermiogenesis has obviously been disrupted. This abnormality is compensable and seldom occurs in very high numbers except in accumulators or when examining frozen thawed sperm.

Vacuoles and Teratoids (VT): Nuclear vacuolation occurs during spermiogenesis and can be caused by such insults as extreme temperatures or stress. The abnormality is more commonly observed in Bos indicus cross bulls than in Bos taurus breeds (Felton-Taylor et al., 2018)Some bulls are predisposed to this condition (perhaps due to a hormone imbalance in the testis) following a stress event (Barth, 2013a; Callaghan et al., 2016).Three forms of vacuolation occur; large confluent vacuoles, diadem defect, small apical vacuoles.

Large confluent vacuoles (LCV) or craters can be so large as to be a "bite" size piece missing from the side of the head. This abnormality may occur after disruption to spermiogenesis, for example following a ruminal acidosis (Callaghan et al., 2016). It has also been reported as an inherited abnormality in a Santa Gertrudis herd (Olley, 2001). Smaller craters were also seen on other spermatozoa in the ejaculate. Bulls with a high percentage of this abnormality were infertile. Canadian studies concur with this effect finding levels of >20% reduce pregnancy rates(Barth, 2013a).

Apical vacuoles are commonly associated with the diadem defect or with multiple small vacuoles scattered throughout the nucleus. Unlike LCV or diadem however, they appear to be more transient than the other forms. Ejaculates with high numbers of apical vacuoles (80%) have reduced conception rates and in an IVF study no sperm with these vacuoles were observed inside the zona pellucida(Barth, 2013a)

The diadem defect, an arrangement of vacuoles along the equatorial region of the sperm appears a serious cause of infertility in the bull. Fluctuations in the prevalence of this defect occurs between ejaculates (Larsen and Chenoweth, 1990) with stress being a predisposing factor.

A high incidence of the vacuole defect, greater than 60% (Pilip et al., 1996; Thundathil et al., 1998) is known to cause severe reduction in fertility. There is debate as to whether this abnormality is compensable as some sperm with this defect could bind to the zona and did initiate fertilization (but it could not be determined if this fertilization produced viable zygotes)(Thundathil et al., 1998). Further, most of the spermatozoa with this abnormality did not reach the zona. However, (Pilip et al., 1996) found that sperm with multiple nuclear vacuoles had a reduced ability to fertilize ova.

In view of this uncertainty nuclear vacuoles are given a threshold level of not more than 20%.(Thundathil et al., 2001)

Abnormal DNA condensation. This condition is included in the Vacuole/Teratoid category under the ACV bullreporter system. It cannot be detected by light microscopy with routine staining techniques. It can be detected by SCSA- a flow cytometric assay that uses the metachromatic properties of acridine orange to measure the susceptibility of chromatin to denaturisation(Barth, 2013b). Feulgen stained semen smears also permit examination of abnormal DNA condensation under x1000 phase contrast or DIC microscopy. Feulgen and SCSA methods correlate and both enable assessment of affected sperm (Dobrinski et al., 1994). Advances in DIC microscopy also now permit the examination of abnormal DNA condensation in the form of pale centres but not as clumping. When this or pale centres are considered the major reason for bull sperm falling below threshold Feulgen staining is often completed as a check.

Rolled Head- Nuclear Crest- Giant Head Syndrome. This abnormality is also included under the vacuole/teratoid category as it is uncommon. It is thought to be an inherited condition. The prognosis for recovery is very poor (Barth and Oko, 1989). The number tolerated in the ejaculate is at 20% because of the ability to penetrate the zona pellucida but the inability to produce a viable embryo. Reports upon its effect, when present at 20-30% of the ejaculate, on conception rates vary between 27-74% (Barth, 2013a)

Teratoid Spermatozoa. These are spermatozoa that are so grossly abnormal in structure as to be barely recognisable as a sperm cell. The sperm nucleus varies from normal to grossly misshapen, may be vacuolated and the tail is often coiled up completely and lies superimposed on the head.These cells are indicative of severe disturbance to spermatogenesis and spermiogenesis. They often occur at very low levels in the spermiogram (1%) but when seen at higher levels the prognosis is poor. There should be no more than 15% of this type of sperm in an ejaculate and they should be associated with at least 70% normal sperm

<u>Loose Heads /Tail abnormalities (HT)</u>**:**

Loose/Detached Heads. This is a problem that can arise with testicular degeneration or hypoplasia, inflamed ampullae or epididymis, heat stress and more usually, as a sign of a “rusty load”. If the motility is low in the initial crush side motility assessment of the semen then further ejaculates (up to 3) should be taken so that sperm that may have “accumulated” in storage can be eliminated and a representative sample collected. In the representative sample, fertility can be related to the percentage of detached heads found: the bull can still be considered “fertile” with 30-40% of this defect, but if the ejaculate contains 70% of this abnormality the bull would have severely decreased fertility. This is considered to be a minor abnormality and some latitude is allowed as it is considered to be a compensable effect; these sperm cannot participate in fertilization, as they cannot swim up the female tract.

The decapitated head defect has been reported in Guernsey and Hereford bulls. This may be an inherited problem. It can be differentiated from detached loose head by the large number of vigorously moving tails in the fresh specimen and the presence of the proximal droplet still attached to the tail. This trait when it occurs affects 80-100% of sperm in the ejaculate.

Principal Piece/Tail Defects. These are seldom seen in high numbers and may be caused by temperature shock or stress event during passage through the epididymis (Barth and Bowman, 1994), therefore levels of this defect may decrease after 8-11 days. Levels of 30% are acceptable with 70% normal sperm as this is a compensable abnormality.

Midpiece Defects (MP): Mid piece and tail defects are generally considered to be compensable traits as the spermatozoa with these traits cannot reach the fertilization site due to the effects upon forward motility.

Distal Reflex Midpieces. This by far the most common defect seen in bull ejaculates(Perry et al., 2002; Menon et al., 2011) not to be confused with a simple bent tail as the midpiece is also involved in the bend. This defect can occasionally arise as an artefact due to prolonged contact with a hypotonic solution (e.g. Nigrosin-Eosin stain), cold-shock, or other environmental stresses. It is usually of a transient nature with recovery likely within 16 days. The presence of a cytoplasmic droplet at the tail bend identifies the problem as one occurring mainly in the distal half of the cauda epididymis. The prognosis varies with circumstance and the types of other abnormalities present. Where it occurs with abnormalities such as a fracture at the tail bend, aplasia of the midpiece or Dag-like defects there may be an underlying cause such as disturbed spermiogenesis. Some bulls have a predisposition for this defect with fluctuations in the percentage of affected spermatozoa throughout the year. Up to 30% of this abnormality is tolerated in the ejaculate as these cells display reverse motility and would therefore be unable to penetrate the zona pellucida so other normal cells would be able to participate in ovum fertilization (Barth and Oko, 1989).

Dag-like defect or doubly bent tails. This can be an inherited defect with a serious effect upon fertility when present in large numbers (>50%)(Koefoed-Johnsen et al., 1980; Barth and Oko, 1989). It can reflect disturbance in the testicle or epididymis and is not normally present at >4%. It is a compensable trait as the sperm are not forwardly motile (Perry, 2002). Fertility is therefore only impaired once >30% of this defect is identified in the ejaculate with less than 70% normal sperm. Presences of fractured axonemal elements, with filaments protruding from the sheath are observed.

Segmental aplasia of the mitochondrial sheath. In a case study bull with 90% affected spermatozoa was reported to have normal fertility over 3 breeding seasons ((Barth, 2013b). This would indicate that the condition has little effect on fertility. This condition can be permanent or transient; if the defect is seen to occur in two tests done ten weeks apart it suggests a permanent condition. Gossypol in the diet (Chenoweth et al., 2000) and a viral disease (Bovine Ephemeral fever), (Chenoweth and Burgess, 1972) have both been shown to have an affect on the mitochondrial sheath. If gaps in the midpiece are larger than 3 microns these may result in fractures of the midpiece and sperm showing such severe segmental aplasia are considered under midpiece abnormalities, however, sperm observed with slight gaps (1-1.5 micron) are considered under the normal category (McAuliffe et al., 2010).

Abaxial Tails. The prognosis for this abnormality is determined by the presence or absence of an accessory tail or secondary implantation fossa. Ejaculates containing

60-100% spermatozoa with abaxial tails alone(Barth, 1989) cause no decrease in fertility. However, abaxial tails seen in an ejaculate with other spermatozoa with accessory tails or secondary fossa (Aughey and Renton, 1968) can cause a significant drop in fertility. The cause of this difference lies in the formation of the tail within the spermatid. Tail formation begins with the migration of the proximal and distal centriole to the base of the nucleus. The distal centriole gives rise to the tail with the proximal centriole forming the neck of the midpiece. Normally in spermatids replication of the centrioles is suppressed so that one flagella is formed. Lack of this suppression may allow the formation of additional tails. The presence of additional fossa and/or tails therefore may indicate the presence of additional centrioles. These structures are critical to the separation of chromosomes during the first cleavage of the ovum. This being the case, abaxial tails should not be considered a defect if present on their own. However, if abaxial tails are present at relatively low numbers (12-20%) with >17% accessory tails the bull would be considered of questionable fertility (Barth and Oko, 1989). Abaxial tails with accessory tails are considered within midpiece defect category, however, within the normal category if present on their own.

Tail Stump Defect. This condition is hereditary inherited via a recessive gene and has a poor prognosis. It is a compensable defect, as the sperm cannot journey to the fertilization site, bulls with 30-40% of this defect have been found to be fertile. It should be noted that care should be taken to differentiate this from detached heads as a cytoplasmic droplet often covers the vestigial midpiece portion.

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