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Effect of non-genetic factors on semen quality in bulls: A review

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Abstract
Artificial insemination is one of the most valuable techniques implemented in bovine reproduction, and it greatly improves breeding efficiency. The semen quality is important in successful conception of the females. The farmers also looking forward for quality semen in order to increase the farm economy. The semen quality is affected by many factors, including the genetic and non-genetic factors. As the genetic factors are heritable traits and are difficult to control in short span of time. Whereas the non-genetic factors can be intervened and managed for better semen quality in bulls. The non- genetic factors are having a significant effect on the bulls performance and the semen quality. The main factors includes THI, season, age and testicular size. The current review compiles effect of different non-genetic parameters on semen quality in buffalo and cattle bulls.

Keywords: Artificial insemination, semen, season, age, bulls

Introduction
India has the largest number of livestock population as compared to our subcontinent. The productive and reproductive performances of livestock depend on their care and management in different stages of life span. They play an important role in the development of socio-economic status of people and the national economy of India which contributes 5.5 percent to Gross Domestic Product at current prices during 2013-14 (Annual Report, DAHD 2014-15). India continued to be the largest milk producing nation in 2014-15 with an anticipated milk production of 146.3 million tonnes, an increase of 6.2 percent over the previous year. The per capita availability of milk increased to 322 gram per day which is more than the world average. The country's share in world milk production stands at 18.5 percent (Annual Report, NDDB 2014-15).

For improvement of the livestock sector, the breeding programme must maintain superior quality fertile bulls through which semen quality improvement can efficiently be accomplished. Semen quality parameters are considered as most important indicators of fertility of bulls because the poor quality of semen is one of the main reasons for conception failure of the female which affects the production as well as the reproductive life of the Livestock. Due to some natural constraints breeding potential may not be achieved maximum level for buffalo bulls as compared to cattle bulls, as the testicular size is relatively smaller, daily sperm production rate is lower and also epididymal sperm reserve is less in the former species (Suryaprakasa, Narshimha 1993; Sudheer, Xavier 2000) [67, 66]. Macro and microclimate, least management practice, shortage of feed and fodder also affect animal health, growth, production and reproduction. In high humidity climates, thermoregulation mechanism is disturbed due to the poor heat regulation mechanism in the presence of less number of sweat glands in buffalo that has a deleterious effect on normal spermatogenesis. The complex cycle of spermatogenesis is controlled by various hormones and thermoregulation mechanism. The scientific literature available on these aspects has been reviewed based on the objectives of the present study and it is presented in this chapter.

Artificial insemination is one of the most valuable techniques implemented in bovine reproduction, and it greatly improves breeding efficiency. Presently, there are 50 semen stations owned by Governments, Cooperatives, NGOs and private companies together produced around 95 million frozen semen doses during 2014-15. Under NDP-I, three more semen stations were added to the list (NDDB, 2014-15). The demand for semen doses for artificial insemination has been growing continuously in the country. In the last five years, the demand for frozen semen doses has increased by an average of 12 percent per year. It is projected that the demand for frozen semen in India will be around 100 million doses by 2016-17 and 140 million doses by 2021-22. The dairy farmers are also now looking for quality semen. Presently around 25 percent of the breedable bovine females are covered under AI. In the case of buffaloes out of about 54.5 million breedable buffaloes in the country, barely 15-20 percent are being artificially inseminated, while 80-85 percent are covered by natural service mostly by scrub bulls. The key purpose of frozen semen preparation is for harvesting the maximum amount of high-quality sperm from genetically superior bulls for its use in AI, as more than 60 percent of genetic improvement occurs through a selection of bulls.

Different non-genetic parameters affecting semen quality
Temperature Humidity Index (THI)

Heat dissipation is an essential mechanism for the maintenance of normal body temperature and normal functioning of body organs (Verma and Hussain, 1991) [69]. High ambient temperature has an adverse effect on health and sexual behaviour of animals like puberty, testicular degeneration and reduces percentages of ejaculate, normal and fertile spermatozoa in males, due to disturbances of sexual activity. Reduced libido might result in higher reaction time and subsequently, total time for successful ejaculation also increase, thus having an ultimate effect on the production of sperms productivity and reproductive functions of the animal during summer seasons (Soderquist *et al*., 1996, Abdel-Samee *et al*., 1997 and Vogler *et al.*, 1991) [65, 1, 70]. Temperature humidity index is a common indicator of heat stress in an animal, used to identify the performance of animal which is reared in tropical and subtropical climatic conditions. Buffaloes show a poor thermal tolerance power due to the presence of less number of sweat glands and underdeveloped thermoregulatory mechanism (Marai and Haeeb, 2010) [48]. The environmental factors such as extreme heat waves also have a deleterious effect on reproductive performance of animal (Hansen, 2009) [25]. Hence they are unable to get rid of excess metabolic heat and are susceptible to heat stress.

In dairy cattle, conception rate and pregnancy rate were found to be decreased above THI of 72, while in buffalo this trend was observed above threshold THI of 75 percent (Dash *et al.*, 2013) [21]. A negative correlation between reproduction traits of cattle and buffalo with THI was observed and animal shows the adverse effect when the THI cross threshold value. The poor reproductive performance in buffaloes, especially during the summer months, is due to their dark skin and sparse coat of body hair which absorb more heat along with poor heat dissipation and inefficiency in maintaining the thermoregulation under high environmental temperature and relative humidity (RH). THI is negatively correlated with a temperature gradient and positively correlated with the ocular temperature of the animal in different environment conditions (Menegassi *et al.,* 2015) [50]. The temperature gradient is negatively correlated with ocular temperature and rectal temperature (Menegassi *et al.*, 2015) [50]. A number of temperature humidity index (THI) models are used to measure the degree of heat stress affecting fertility traits of an animal by using temperature and relative humidity (Yousef, 1985) [72]. Kunavongkrit *et al*. (2005) [44] reported that climate factors such as humidity and temperature can cause thermal discomfort, resulting in a decrease in food intake, interference with normal spermatogenesis and semen quality traits. The ambient temperature and THI showed a positive correlation with biochemical attributes. Which indicate that the temperature and THI of the season significantly affect the semen biochemical attributes of Bhadawari bulls (Pandey *et al*., 2014) [53]. Observed significant negative correlation only with plasma membrane integrity (−0.17; $P<0.05$), and for all others seminal parameters, the correlations with THI were not significant ($P>0.05$) in water buffalo bulls. Rhouma and Mrad (2012) [4] found a significant effect of ambient temperature on the scrotal surface and non-significant effect of season on the breed, age and testicular temperature in bulls.

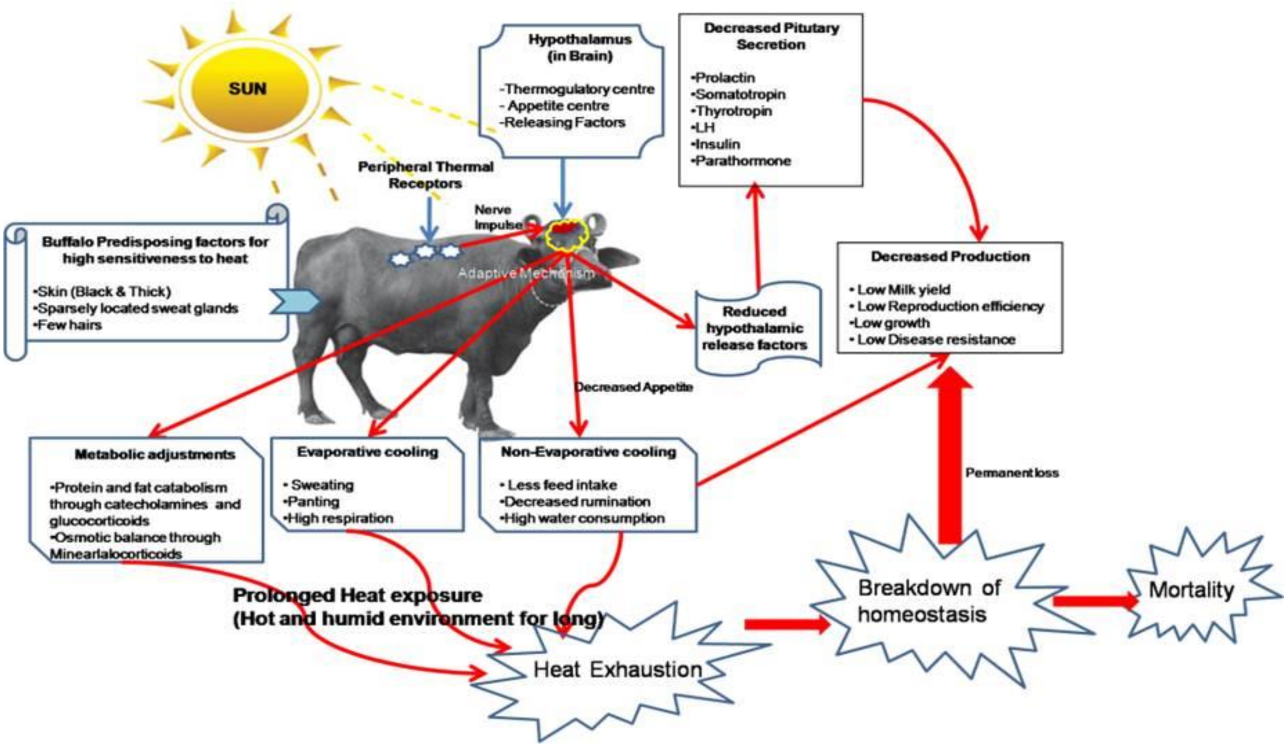

Fig 1: Physiological mechanism during heat stress in buffalo (**Source:** http://www.buffalopedia.cirb.res.in)

Table 1: Classification of zones based on THI values in cattle with THI model

THI	Stress level	Symptoms in cattle	Symptoms in buffalo
<72	none	None Optimum productive and reproductive performance	Optimum productive and reproductive performance
72-80	mild	Dairy cows seek for shade, increase in respiration rate and dilation of blood vessels	Elevation in rectal temperature and respiration rate
79-88	Moderate	The increase in respiration rate and saliva secretion. Reduction in feed intake and water consumption. Body temperature is increased and reproductive performances are severely affected in cattle	Respiration rate is significantly increased. Dry matter intake of buffalo is decreased and the ratio of forage to concentrate intake is decreased. Water intake in buffalo is significantly increased
89-98	severe	There is a rapid increase in respiration and excessive saliva production. The reproductive performances in animals are significantly decreased	Excessive panting and restlessness are observed. Rumination and urination are lowered along with a negative impact on reproductive performances in buffaloes
>98	Danger	Heat stress is extreme and cows may die	Heat stress is extreme and buffaloes may die

Source: Armstrong, D.V. (1994)

Season

Buffalo is considered as a seasonal breeder among dairy animals and the maximum breeding activity occurs during autumn and winter while the lowest is in summer (Heuer *et al*., 1987) [29]. The season has a direct influence on libido, quality and freezability of semen. In tropical and subtropical climatic conditions there is a huge impact on production and reproductive performances of the animals (Bhakat, 2014) [9]. Some research workers have given conflicting report about the effect of seasons on semen quality in bulls. Gupta *et al.* (1978) [24]; Bhosrekar (1992b) [13]; Prajapati, (1995) [54] reported ill effects of heat stress, while others observed (Bhat *et al*., 2004) [11] similar findings during the winter season; whereas such effects had been reported in the spring season by Sengupta *et al.* (1963) [58]. Raviurugan *et al.* (2003) [57] and Bhat *et al.* (2004) [11] reported monsoon proved to be the best season for production of quality semen in bulls. There are a number of reports available about the impact of heat stress on libido and semen production of the breeding bulls. Bhakat *et al*. (2011) [7] reported hot-dry and hot-humid season were unfavourable for production as well as reproduction. The information regarding the effect of seasons on seminal characteristics in dairy bulls has been reported by various researchers. There are reports showing both significant effect of season (Goswami *et al*., 1991 and Singh and Raina, 2000) [23, 64], as well as non-significant effect (Mathur *et al*., 2002; Helbig *et al*., 2007) [49, 27] of the season on semen quality parameters.

Menegassi *et al.* (2015) [50] have studied bull reproduction in association with infrared thermography. The regional variation could be the reason for the difference in their reports. In general, summer exerts a comparatively more adverse effect on the overall quality of semen. The summer stress affects the normal reproductive function multi-dimensionally, by reducing feed intake, inhibiting release or response to GnRH, FSH and LH. All these hormones together control the spermatogenesis. The LH which is the most important for spermatogenesis is inhibited by increasing the level of plasma corticosteroids during heat stress. The reduced secretion of thyroxine and further reduction in feed intake may also be a reason for the reduction in sperm quality during summer. Due to extreme heat stress, bulls get physically exhausted and their reduced libido might result in higher reaction time and subsequently, the total time for successful ejaculation also increases, thus having an ultimate effect on the production of sperms (Mandal *et al*., 2000) [45].

Bhakat *et al.* (2015) [8] reported that season had no significant effect on ejaculate volume, mass activity, total sperm output and pH of the semen, whereas it had significant (P<0.05) effect on initial motility, non-eosinophilic count and acrosome integrity of sperms and highly significant (P<0.01) effect on HOST, sperm concentration, sperm abnormalities and osmolality of semen. They reported that the semen qualities were optimal during winter, poor during summer and intermediate during the rainy season. Rajoriya *et al.,* (2015) [55] reported significant (P≤0.01) effect of season on volume in Murrah bulls. Bhattacharya *et al.* (1978) [12] and Mandal *et al.* (2000) [45] reported highest semen volume in Murrah bulls during the summer season and lowest during the winter season. However, Rao *et al.* (1991) [56] and Ravimurugan *et al.* (2003) [57] obtained highest ejaculate volume during the rainy season. Sengupta *et al.* (1963) [58] and Singh and Singh (1993) [62] reported highest semen volume during the spring season. No significant seasonal difference was observed in ejaculate volume which is in agreement with the findings of Oloufa *et al.* (1959) [52] in Egyptian buffalo bulls; Tomar *et al.,* (1966) [68] in Murrah buffalo bulls and Koonjaenak *et al.,* (2007) [41] in Swamp buffalo. Factors such as the age of the animal, differences between species, the number of specimens, the level of nutrition, management practice and environmental conditions etc. may be responsible for the differences in results.

Age

In general, scrotal circumference (SC), scrotal shape score, scrotal neck perimeter, and testicular size (length, width and volume) increase with age. However, there was no significant effect of age on sperm concentration, motility, major and total defects (Brito *et al*., 2002) [14]. A significant effect of age of bulls on the percentage of dead spermatozoa was observed, the values being higher in old than young and adult bulls. Although buffalo bulls breed the year round, conflicting reports have been published about semen quality and volume at different ages and during various seasons of the year. Bhakat (2011) [7] reported that all semen traits were significantly affected by age, except sperm concentration. The ejaculate volume (ml) significantly enhanced with the increasing age (year) of bulls (4 to 6 years) but decreased again for older age groups (>6 years). The mass activity of semen significantly increased as the bull matured but decreased again for the older age group. Mass activity was highest in 4 to 5 year age group bulls and lowest in <4 and 5 to 6 year age group bulls. The percentage of progressively motile sperm was recorded in >6-year bulls. The highest and lowest sperm concentrations were recorded for the bulls in 4 to 5 year age group and <4 year age group, respectively.

Sperm concentration per ejaculate was higher in 5 to 6 year age group bulls.

Younis *et al*. (1998) [71] studied in18 Nili-Ravi buffalo bulls of different ages ranging from 3 to 15 years. The Ejaculatory volume was higher (P<0.05) in adult and old than young bull during the low (May-July) than the peak (September-November) breeding season. Mass activity was significantly higher (P<0.05) in the peak than the low breeding season. Sperm concentration did not differ among bulls during two seasons. Total sperms per ejaculate were higher (P<0.05) in semen collected from adult and old than in young bulls and in the low than the peak breeding season. Higher (P<0.05) percentage of motile spermatozoa was seen in ejaculates collected from young and adult than old bulls and during the peak than the low breeding season. Percentage of dead spermatozoa was higher (P<0.05) in semen collected from old than young and adult bulls and in the low than the peak breeding season. Ejaculates collected from old bulls had higher number of abnormal spermatozoa than those from young and adult bulls and in the low number of abnormal spermatozoa in peak breeding season.

Reported that normally the total sperm per ejaculate volume increase with age up until 7 years of age and then declines and scrotal circumference increased rapidly with age up to 48 months, and more slowly thereafter. Younis *et al*. (1998) [71] reported in Nili-Ravi buffalo bull the freezability of semen from adult (6-8 years) buffalo bull was better than that from young (3-4 years). Silva – Mena *et al*. (2000) [60] studied the sexual behaviour and pregnancy rate in *Bos indicus* bulls (9 Brahman & 6 Nellore bulls) and reported a significant correlation between libido and serving capacity, and age (r = 0.78, P>0.01 and r = 0.56, P>0.05 respectively).

In HF bulls, reported that breeding efficiency declines with the age and that fertility (as measured by services required per conception) highest with yearling bulls. On the other hand, Amstalden (1994) [2] studied, qualitative and quantitative characteristics of bubaline semen, in which 229 ejaculates collected from 10 crossbred Murrah buffalo bulls and reported that age of bulls had no significant effect on ejaculate volume, sperm motility, viability, and concentration, semen pH, and incidence of sperm abnormalities. Javed *et al*. (2000) [31] conducted a study 16 Nili-Ravi buffalo bulls which were divided into four age groups, including three healthy (less than 5, 6-10, and more than 11-year-old animals), and one abnormal group (6 to 10-year-old bulls having poor semen quality). Overall semen volume was 4.67±1.62 ml and showed a non-significant difference between age groups, Overall mass activity score was 2.65±1.03 and was lower (P<0.05) in more than 11-year-old bulls. However, it was lower (P<0.05) in the abnormal than in healthy groups Overall sperm motility was 56.89±0.65% and showed no difference in healthy groups, but was lower (P<0.05) in the abnormal group. Overall sperm concentration was 1.00±0.50 billion/ml and was higher (P<0.05) in less than 5-year-old bulls. However, it was lower (P<0.05) in the abnormal group.

Brito *et al*. (2002) [14] found a significant effect of age on the scrotal circumference (SC), scrotal shape score, scrotal neck perimeter and testicular size (length, width and volume). In general ejaculate volume, a total number of spermatozoa and number of viable spermatozoa increase with age and non-significant effect on sperm concentration, motility, major and total defect. Kiani *et al*. (2014) [39] studied, physical characteristics of semen in different age group (<5 years, 6-8 years and > 8 years) of Khundi buffalo bulls and found that higher ejaculate volume in adult than young followed by older, in colour no significant change was observed, higher pH observed in older and lower in young, mass activity was higher in adult than older and youngs, sperm motility was lower in old bulls than in young and adult bulls.

Testicular size

In recent decades farmer's interest in breeding for conservation and transmission of superior Murrah buffalo germplasm has increased (Singh, 2009) [63]. Therefore good quality frozen semen are required from high genetic source to achieve maximum production (Kumar *et al*., 2014) [42]. In that scrotal circumference is one of the most important criteria for the breeding soundness evaluation of bulls. Scrotal circumference and testosterone have highly significant (P<0.01) positive correlation with ejaculate volume, MA (Mass activity) and Individual motility (IM) and has a negative correlation with head, midpiece, and tail abnormalities. The results indicated that SC and testosterone are useful indicators of sperm production and important criteria for selection of high genetic potential young Murrah bulls (Kumar *et al*. 2014) [42] studied in Aberdeen Angus, Hereford, Poll Hereford and Charolais breeds. Singh *et al*. (2014) [53] found that no significant correlation between First service conception rate (FSCR) and scrotal circumference. Similarly, no consistent relationship existed between sperm concentration and scrotal circumference. Out of six post-thaw seminal components (total motility, progressive motility, viability, HOST (%), total abnormality and concentration), only total motility had a high significant (P<0.01) correlation with FSCR (r=0.694). Kastelic *et al*. (2001) [36] reported that scrotal circumference had a positive linear regression with the percentage of progressively motile sperm and epididymal sperm reserve but negative linear regression with the incidence of primary sperm defects.

Nutrition

For better quality of semen production, proper management is the most important thing but very limited information is available about proper feeding and watering of buffalo bulls. Green fodder is one of the main sources of nutrients to fulfilling the requirement for the most part of the animal diet. The quality of fodder varies according to the seasons, most of the fodder seasonally available throughout the year. In terms of crude protein (CP) and total digestible protein, little information is available for production. Khan *et al*. (1997) [38] studied on different sources of crude protein and energy on the seminal attributes in Nili-Ravi buffalo bull and found that diet has a significant effect only on the number of doses but within diets, seminal attributes differed significantly except individual motility. Thermoregulation is a most important factor related to feeding efficiency because it is linked with energy metabolism and energy outflow (Herd *et al*., 2004) [28]. Thus, thermoregulation could be explored for this purpose. Spermatogenesis is extremely affected by post-weaning dietary energy. After weaning bulls which are fed mostly with moderate-energy diets (100% forage) have a greater significant effect on reproductive functions as compared to other animals those are fed high energy diets (80% grain, 20% forage; Coulter *et al*., 1987) [15]. It is not clear why feeding higher energy diets decrease reproductive capacity. Perhaps excess fat in the neck of the scrotum and (or) scrotal tissues insulates the testes and increase scrotal/testicular temperature, thereby decreasing sperm production and seminal quality

(Coulter *et al*., 1997) [17]. Kumar *et al*. (2015) [43] reported a significant effect of dry matter on testosterone concentrate (ng/ml) and higher energy (10 and 20%) on sperm concentration in Murrah bulls, however, non-significant effect on body weight, average daily weight gains and scrotal circumference.

Thermoregulation

The testicular thermoregulation mechanism plays an important role in normal spermatogenesis, which maintains the fertility of mature bull, alteration of thermoregulation mechanism have a deleterious effect on spermatogenesis (Marai *et al.,* 2008 and Hansen, 2013) [47, 26]. There are several features that regulate testicular temperature. Scrotal skin is usually thin, relatively hairless with numerous subcutaneous blood vessels, all of which promote heat loss, whereas presence of less number of sweat glands in buffalo which has affect productive and reproductive functions such as poor breeding efficiency, late onset of puberty, changes in animal temperament, poor semen quality, less intake of feed and fodder. For good quality of sperm production, the scrotal surface temperature must be maintained at 2 to 5 ^{0}C lower than core body temperature (38 ^{0}C) of breeding bulls. It is well defined that for maintenance of scrotal thermoregulation, various part of reproductive organs (pampiniform plexus or testicular vascular cone along with spermatic cord) function as a counter- current heat transfer system from the warm blood in the testicular artery to the cooler blood in the testicular venous system with contraction and relaxation of dartos and cremaster muscles (Setchell, 1978; Coulter and Kastelic, 1994, 2007) [59, 19]. Evaporation is also another mechanism for maintenance of testicular temperature due to the presence of sweat and sebaceous glands on the scrotal surface.

At high temperatures, these glands are more active (Bearden *et al*, 2004) [3]. The third mechanism is based on creating distance from the body and dilatation of scrotal muscles at higher ambient temperatures, therefore, locating the testes further away from the body and thus letting it cool down (Bearden *et al*., 2004) [3]. The scrotal skin of the zebu bull is thin and hairless and can stretch allowing the many vessels to dilate to increase heat loss. The proportion of defective spermatozoa will be higher when testicular temperature increases. If the elevation of the temperature in the testes is very high, this could even stop spermatogenesis (Bearden *et al*., 2004) [3]. The testicular temperature can be measured by introducing sensors into the gonads because testicular thermoregulation is directly related to blood flow in the testicular artery. This procedure is considered invasive and risks to the animal. Coulter *et al*. (1988) [16] evaluated a non-invasive method for measuring testicular temperature by the use of infrared thermography and showed no difference between this method in relation to sensors. Furthermore, an indirect evaluation of testicular thermoregulation can be performed by infrared thermography, Doppler ultrasound examination of the spermatic cord. There are a few studies evaluating a testicular temperature in cattle (Coulter *et al*., 1997; Kastelic *et al*., 1996) [17, 32, in goats (Coulter *et al*., 1988; Kastelic *et al*., 1997) [16, 17], in humans (Gold *et al*., 1977) and in horses (Janet *et al*., 2015) [30].

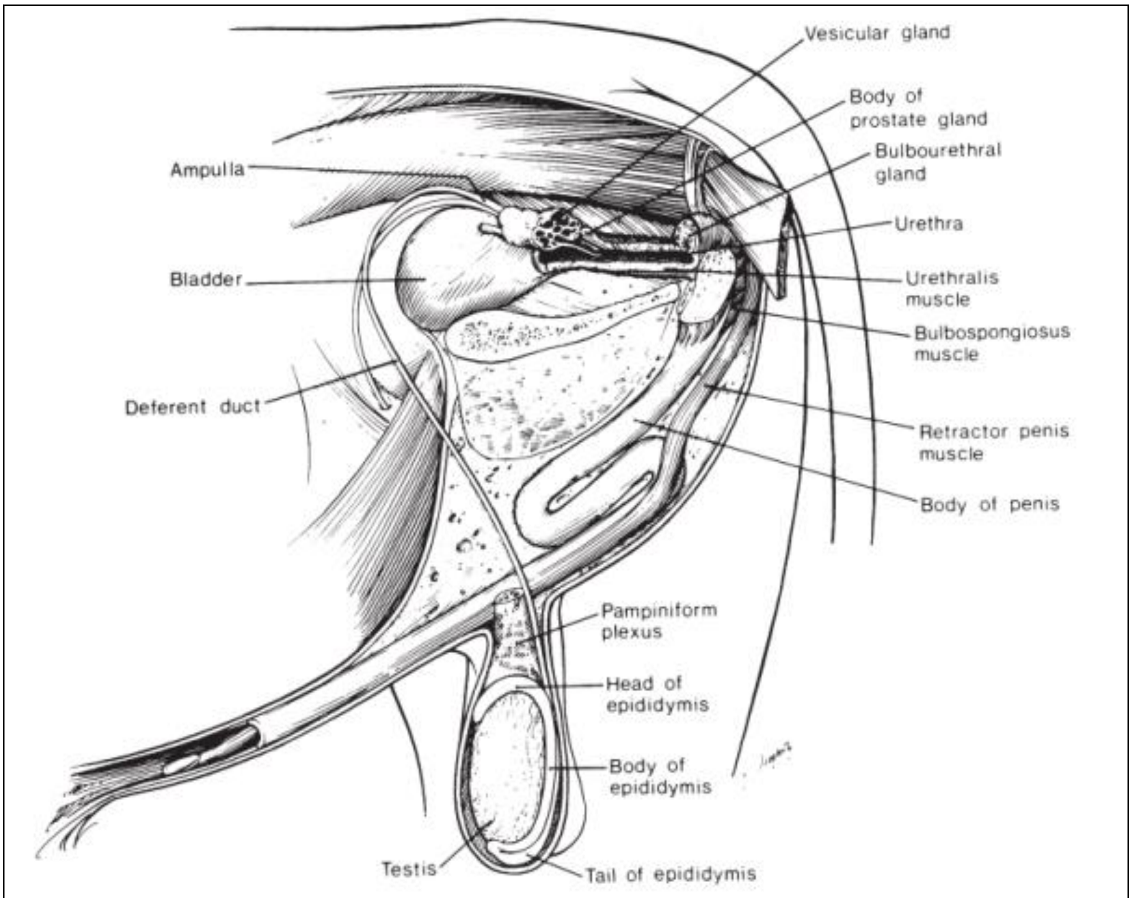

Fig 2: Male reproductive organ of bull (**Source:** Draaisma, 2015)

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