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RESEARCH ARTICLE
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Association between the seminal vesicle weight and certain steroids in buffaloes (*Bubalus bubalis*)

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Abstract

Aim: Present study was carried out to study the association of seminal vesicle weight with steroidal (testosterone and estrogen) concentration in buffalo bulls.

Materials and Methods: A total of 30 pairs of seminal vesicles were collected from buffalo bulls (irrespective of age and breed) and categorized into group I (<5 gm), group II (5-8 gm) and group III (>8 gm) on the basis of mean seminal vesicles weight for establishing relationship.

Results: The mean concentration of testosterone in seminal vesicle fluid showed, significantly ($p<0.05$) higher value in group III (0.54±0.09 ng/ml) compared to group I (0.33±0.03 ng/ml), however, difference between group III and II (0.51±0.05 ng/ml) was non-significant. Similarly, mean estrogen concentration also showed a significantly ($p<0.05$) higher value in group III (16.31±4.70 pg/ml) compared to group I (2.51±1.09 pg/ml) but values in group III and I were non-significant with group II (8.99±2.55 pg/ml).

Conclusion: Present study indicated positive correlation between seminal vesicle weight with testosterone ($r=0.365$, $P< 0.05$) and estrogen ($r=0.528$, $P<0.01$) concentration respectively in seminal vesicle fluid.

Keywords: buffalo bulls, estrogen, seminal vesicle fluid, seminal vesicle weight, testosterone

Introduction

Seminal vesicles (vesicular gland) are firm and lobulated structures located adjacent to the neck of bladder and lateral to ampulla [1]. As age of the animal increases, the size of seminal vesicles also increases in terms of its biometry, thus there is a positive correlation between age of animal and size of seminal vesicles [2]. Furthermore, the effect of age and breed on the seminal vesicle's weight and its secretory activity has been reported [3]. Interestingly, its secretory activity is dependent on the level of androgen in male reproductive tract.

In mammals, The HPG axis drives reproduction: Hypothalamus secretes Gonadotrophin Releasing Hormone (GnRH), GnRH stimulates the gonadotroph cells of pituitary to secrete Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH), and in turn these two hormones regulate the gonadal function in both sexes [4]. Steroids like testosterone and estrogen are no longer considered male specific hormones as both hormones are important in male and female. In males, testosterone produced by Leydig cells is converted to estradiol in the sertoli cells under the influence of aromatase [5]. Also in brain, the androgen is converted to estrogens by an enzyme aromatase cytochrome P450 (P450arom) which has a central role in organizing neuroendocrine function and regulates a variety of reproductive and social behaviours [6]. Mammalian semen is known to contain a big variety of chemical elements [7] produced under the influence of androgens. Their influence on spermatozoa viability has been extensively studied in animals as well as in humans. The P450arom protein is highly conserved in its function, if not its peptide sequence [8] and is expressed in the brain of all vertebrates studied, including amphibians, birds, fish, and reptiles [9]. A significant correlation between the growth and the secretory activity of the seminal vesicles controlled by the level of androgens: testosterone, androstenedione and dihydrotestosterone, has been reported [10]. Presence of high levels of estrogen has been reported in semen compared to blood plasma, even after frequent excessive ejaculations [11], thus it suggests that estrogen is being secreted into semen mostly from accessory glands. Currently, researchers indicate that germ cells also synthesize estrogen, and possibly serve as the major source of estrogen in the male reproductive tract [12]. Together with the existence of a functional aromatase, the intracrine role of estrogens is important in production of immature germ cells as well as during ? nal steps of spermatozoa maturation [13]. It was also demonstrated that during fetal and neonatal

Table-1. Mean ± SE of testosterone concentration in three seminal vesicle weight (gm) groups in buffaloes *invitro*

Parameters	Group (mean seminal vesicle weight)			Mean total
	(<5 gm)(Group I)	(5-8 gm)(Group II)	(>8 gm)(Group III)	
Seminal vesicle fluid testosterone (right) (ng/ml)	0.31±0.02^{bA} (0.20-0.39)	0.52±0.06^{aA} (0.20-0.83)	0.49±0.09^{abA} (0.23-0.94)	0.45±0.04 (0.20-0.94)
Seminal vesicle fluid testosterone (left) (ng/ml)	0.34±0.05^{bA} (0.11-0.58)	0.50±0.05^{abA} (0.27-0.90)	0.58±0.09^{aB} (0.32-0.99)	0.47±0.04 (0.11-0.99)
Pooled Seminal vesicle fluid testosterone (ng/seminal vesicle)	0.65±0.07^{b} (0.32-0.95)	1.02±0.11^{a} (0.48-1.65)	1.07±0.19^{a} (0.55-1.92)	0.92±0.07 (0.32-1.92)
Mean Seminal vesicle fluid testosterone (ng/ml)	0.33±0.03^{b} (0.16-0.48)	0.51±0.05^{a} (0.24-0.82)	0.54±0.09^{a} (0.27-0.96)	0.46±0.04 (0.16-0.96)

Mean with different superscripts in a row (a, b, c) and in a column (A,B) differ significantly at $p<0.05$ and $p<0.01$ respectively. On multiple comparison, the mean differ significantly between I vs II and I vs III at $p<0.028$ and $p<0.035$ respectively. Group-1: (n=9), Group-2: (n=14), Group-3: (n=7), Group-4: (n=30)

life, estrogens are involved in control of gametogenesis, promoting germ cell and seminiferous tubule development and in the regulation of fetal Leydig cell steroidogenesis [14]. Testosterone either alone or with estradiol 17 β has been reported to maintain sperm motility for 14 days after castration [15]. However, there are few studies which have shown concentration of steroids in seminal vesicle tissue. In buffaloes, overall mean testosterone concentration in seminal vesicle flushed and squeezed fluid has been reported as 0.32±0.06 ng/ml and 0.24±0.03 ng/ml respectively [16]. In Holstein- Friesian bulls, estrogen concentration has been reported in tissues of seminal vesicle as 0.008 ± 0.002 ng/gm [17]. The estrogen with lower dose of 2 microgram/ml showed a beneficial effect on motility and acrosomal integrity of bull sperm in-vitro [18].

The information regarding the testosterone concentration in seminal vesicle fluid and in tissues of buffalo bulls [16] and levels of estrogen in bulls [17] has been previously reported. In this study, we have established an association between the mean seminal vesicle weight and its hormonal content as an indicator of fertility in buffaloes.

Materials and Methods

Ethical approval: samples were collected from local slaughter house where animals are sacrificed with Government approval. We have not sacrificed any animal for our study so there are no ethical issues in this study.

Climate and experimental animals: Geographically, Bareilly is located at 28° 10' North latitude and 78° 23' East latitude at an altitude of 172 meters above the mean sea level. Bareilly is known to have moderate climate. Summer season goes up to 40° C while winter goes down up to 8° C. The rainy season starts in June and extends up to September with humid and warm conditions. Thirty pairs of seminal vesicle were collected from buffaloes' sacrificed at local abattoir, irrespective of age, breed and body weight. Immediately after sacrifice (within an hour), the seminal vesicles along with other tissues were collected and ligated at the base of the seminal vesicular duct to prevent fluid loss and finally placed in a polythene bag (with sealing device) and transferred into the thermos flask containing ice cubes and then transported to laboratory. In laboratory, the seminal vesicles were cleaned and then carefully cleared the tissue debris, fatty tissue, fascia and other part from seminal vesicles. Then organs were cleaned again (with normal saline, 0.9% NaCl, GR Grade, Merck, India), soaked and dried with tissue paper before processing. The gross morphological parameters of seminal vesicles (right and left separately) were recorded.

Seminal vesicles' (right and left) weight was recorded separately before and after squeezing the fluid using electronic balance (A and D Company Limited, Japan). On the basis of mean weight, seminal vesicles were divided into three groups as group I (<5 gm), group II (5-8 gm) and group III (>8 gm).

Seminal vesicle fluid collection: Seminal vesicles (right and left) were squeezed and flushed (using potassium phosphate buffer, 50 mM, pH 7.4).

Squeezing: Each right and left seminal vesicle was squeezed separately and obtained volume of seminal vesicle fluid was collected in the test tube.

Flushing of seminal vesicles: Immediately after squeezing and weighing, each seminal vesicle was incised longitudinally and transversely at all length and width in each seminal vesicular duct and lobule using pointed scissor without separating into pieces. Flushing was done by placing (attaching/fixing in slanted test tube) the organ on the inner aspect of test tube (30 ml), by flowing down 1ml of potassium phosphate buffer (50mM, pH-7.4) drop by drop on all area of each seminal vesicle (right and left separately). Then, fluid obtained after squeezing of right and left vesicular gland separately was mixed with respective flushed fluid. Then, mixed fluid was centrifuged at 3000 rpm for 15 minutes. Supernatant fluid was stored in duplicate in cryovials (eppendorf tube) at -20^{0}C.

Hormone analysis: The concentration of testosterone and estrogen in supernatant fluid was determined by Radioimmunoassay (RIA) kit (Immunotech, France) and radioactivity was counted by COBRA II, Auto Gamma Counter, Packard, as per the standard protocol provided with kit.

Statistical analysis: All the data were statistically analyzed by SPSS 16.0 using one way ANOVA. The

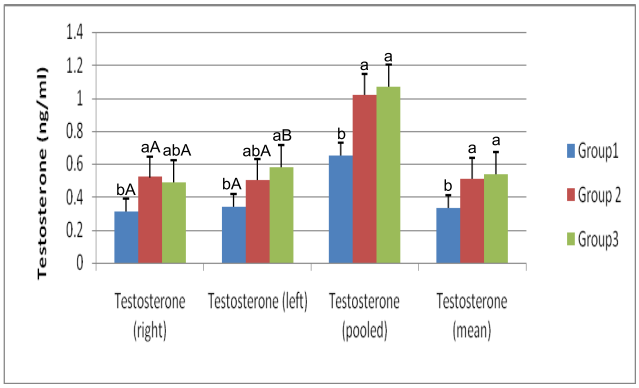

Figure-1. Testosterone concentration in right, left, pooled (ng/seminal vesicle) and mean seminal vesicle fluid in three seminal vesicle mean weight groups in buffalo in-vitro. Values with different letters are statistically significant (P< 0.05)

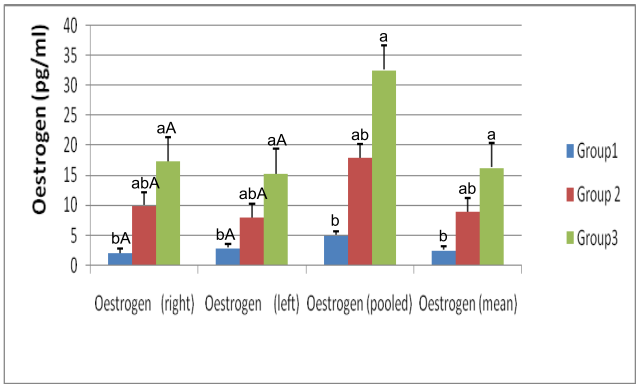

Figure-2. Estrogen concentration in right, left, pooled (pg/seminal vesicle) and mean of seminal vesicle fluid in three seminal vesicle mean weight groups in buffalo in-vitro. Values with different letters are statistically significant (P< 0.05)

Table-2. Mean ± SE of estrogen concentration in three seminal vesicle weight (gm) groups in buffaloes *invitro*

Parameters	Group (mean seminal vesicle weight)			Mean total
	(<5 gm)(Group I)	(5-8 gm)(Group II)	(>8 gm)(Group III)	
Seminal vesicle fluid estrogen (right) (pg/ml)	2.06±0.81^{bA} (0.29-7.62)	9.98±3.18^{abA} (0.42-41.90)	17.34±6.07^{aA} (1.59-42.26)	9.32±2.24 (0.29-42.26)
Seminal vesicle fluid estrogen (left) (pg/ml)	2.96±1.40^{bA} (0.37-13.68)	7.99±2.29^{abA} (1.24-26.04)	15.28±4.04^{aA} (3.03-34.37)	8.18±1.65 (0.37-34.37)
Pooled Seminal vesicle fluid estrogen (pg/seminal vesicle)	5.02±2.19^{b} (0.66-21.30)	17.97±5.10^{ab} (2.14-55.54)	32.62±9.41^{a} (4.62-68.15)	17.51±3.68 (0.66-68.15)
Mean Seminal vesicle fluid estrogen (pg/ml)	2.51±1.09^{b} (0.33-10.65)	8.99±2.55^{ab} (1.07-27.77)	16.31±4.70^{a} (2.3-34.07)	8.75±1.84 (0.33-34.07)

Mean with different superscripts in a row (a, b, c) and in a column (A,B) differ significantly (p<0.05). On multiple comparison, the mean differ significantly between I vs III at p<0.005. Group-1: (n=9), Group-2: (n=14), Group-3: (n=7), Group-4: (n=30)

means were compared by Duncan's Multiple Range test and correlation coefficient among different parameters was also calculated.

Results and Discussion

Testosterone levels in seminal vesicle fluid: Higher values of testosterone in in right seminal vesicle fluid of group II compared to group III and group I (Table-1 and Fig-1) but the difference was significant (p<0.05) only between group I and II. However, testosterone concentration in left seminal vesicle was significantly (p<0.05) higher in group III compared to I and II. Values in group II were not significantly differing from group I or III. The difference was significant (p<0.05) only between group III and I. On the other hand, in pooled and mean seminal vesicle fluid, testosterone concentration showed significantly (p<0.05) lower value in group I compared to group II and III. The variation within group I and II between right and left seminal vesicles were non-significant. However, testosterone concentration was significantly (p<0.01) higher in left seminal vesicle in group III. The intra-assay coefficient of variation was 7.11%. Lower testosterone concentration in flushed and squeezed seminal vesicle fluid of buffaloes *invitro* has been reported [17] compared to our study. Testosterone is the major androgen produced from leydig cells under the influence of LH. Androgens are important hormones for expression of male phenotypes and also have characteristic role during male sexual differentiation during development and also during initiation and maintenance of spermatogenesis [19]. Male reproductive tract, including seminal vesicle are androgen dependent and its secretion are influenced by testosterone. Testosterone levels in the seminal vesicle indicate secretory activity of seminal vesicle.

Interestingly, reproductive organs size (testes and seminal vesicles) are markers of the timing of fertility [20]. In our study, testosterone concentration was significantly (p<0.05) correlated (r=0.365) with seminal vesicle weight. Similarly, in bovines, pituitary gland size increases from, 0.57±0.05gm at birth to 1.77±0.05gm at one year of age [21]. In present study, we found higher weight of seminal vesicle with higher testosterone concentration which influences the secretory activity and thus there will be secretion of several androgen dependent proteins serving as a useful marker of seminal vesicle activity [22]. Similarly, in bulls, significant correlation between the growth and the secretory activity of the seminal vesicles has been reported [10]. Secretions of seminal vesicles involves, proteins that bind to sperm at ejaculation and modify the sperm membrane by removing cholesterol and phospholipids, which may adversely affect the ability of sperm to be preserved [23]. In four year old bull, higher size of seminal vesicles has been reported [2] compared to 2 and 3 year old bulls thus indicating positive correlation between the age and size of seminal vesicles. Similarly in the present study, increase in level of steroids with increase in weight of seminal vesicle might be due to age of animal, as age of animal increases there is increase in size of seminal vesicles.

Estrogen levels in seminal vesicle fluid: There was increase in estrogen concentration from group I to II and to III, but the difference is significant ($p<0.05$) only between group I and III in right seminal vesicle fluid, with overall mean value of 9.32±2.24 pg/ml (Table-2 and Fig-2). Similar trend was also observed in left, pooled and mean seminal vesicle fluid estrogen concentration from group I to II to III with overall mean value of 8.18±1.65 pg/ml, 17.51±3.68 pg/seminal vesicle and 8.75±1.84 pg/ml, respectively. On multiple comparison of overall mean seminal vesicle fluid estrogen concentration showed significantly ($p<0.005$) higher concentration in group III compared to group I. Comparative evaluation of right and left seminal vesicle fluid estrogen concentration showed non-significant changes within groups, however, estrogen was non-significantly higher in right seminal vesicle in group II and III compared to left seminal vesicle. Estrogen, like testosterone plays an important role in both male and female reproduction. However, its role is attributed to estrogen induced accelerated sperm transport [24] as this estrogen regulates spermatozoa motility by up regulation of oxytocin receptor gene and protein [25]. However, the presence of high levels of estrogen has been reported [11] in semen compared to blood plasma, even after frequent excessive ejaculations suggesting that estrogen is being secreted into semen mostly from accessory sex glands.

Similar to testosterone concentration, estrogen concentration also showed significant ($p<0.01$) positive correlation ($r=0.528$) with seminal vesicle weight. The intra-assay coefficient of variation was 17.35%. In seminal vesicle tissue, lower (8 pg/gm) estrogen concentration has been reported [17] compared to estrogen concentration in medium (8.99±2.55 pg/ml) and high (16.31±4.70 pg/ml) weight groups (5-8 gm and >8 gm respectively) seminal vesicle fluid in the present study. The level of estrogen in seminal vesicle indicates its role in spermatozoa transport through reproductive tract which is necessary for obtaining fertilizing ability of spermatozoa., The concentration of both estrogen and testosterone may not only depend on age of animal. Other factors like, environment, management, genetic and nutritional factors could also have contributed.

Conclusion

Seminal vesicles are major accessory sex glands in ruminants and their secretions are dependent on testosterone and its metabolites which are important for enhancing fertilizing ability of spermatozoa. Concentration of both steroids in seminal vesicle secretion can predict the secretory activity of the organ and transport of spermatozoa through reproductive tract. Present study showed the positive relation between weight of seminal vesicles and its hormonal content. The mean concentration of testosterone and estrogen in group III animals (with >8 gm weight of seminal vesicles) were significantly ($p<0.05$) higher compared to group I (with <58 gm weight of seminal vesicles). As already mentioned, the secretions of seminal vesicles are important for obtaining fertilizing ability of spermatozoa. Thus present study showed that seminal vesicle secretion is dependent on level of androgens. Therefore, steroids estimation could be used as a parameter in selection of breeding bull along with other factors.

Authors' contributions

SS planned and carried out research work for his MVSc thesis programme in collaboration with advisory members and guide (SM). GS guided and provided facilities in estimation of steroids using RIA. MRV performed statistical analysis. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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