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EFFECT OF EXOGENOUS ESTROGEN ON SEXUAL BEHAVIOR OF KUNDHI BUFFALO BULL

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ABSTRACT

Estrogen and testosterone are considered as male and female hormones, although, both hormones are produced in both sexes, the estrogen is produced by male reproductive organs in various species. The present study was conducted to determine the effect of exogenous estrogen (Diethylstilbestrol) on sexual behavior and libido of Kundhi buffalo bulls. Four Kundhi buffalo bulls of aged 5-6 years kept under good managemental conditions were chosen for the study, the animals were divided into two groups, group-1 having three numbers of bulls treated as experimental animals and internal control as well, and fourth bull was kept as an untreated external control. 20mg of Diethylstilbestrol was injected intramuscularly to each experimental bull, the intensity of sexual behavior, number of false mounting, degree of intromission and erection were observed in individual bull during semen collection for period of 12 weeks with frequency of 4 ejaculates in a week. The collected qualitative data was converted in numerics and score of 1-3 was given according to intensity of particular parameter. The quantified number of false mounting, sexual excitement, degree of erection and intromission in control versus experimental animals were 1.73 vs 1.90, 1.58 vs 1.77, 1.66 vs 1.52 and 1.58 vs 1.55, respectively. It was concluded that the exogenous estrogens has little impact on sexual behavior in experimental animals. These results indicate that the administrated exogenous estrogen might be utilized by the estrogen receptors present in brain and other various tissue of the body which result in stimulation of sexual excitement.

Keywords: Behavior, buffalo, bull, estrogen.

INTRODUCTION

Livestock is an important component of agriculture which plays a vital role in the economy of Pakistan, the estimated population of buffaloes in Pakistan is 33.7 millions (NLC, 2012). The reproductive health of adult male animals and their germ cells play a vital role in the fertility of animal. Behavior in farm animals is an overt expression of the additive effects of two actions of gonadal steroids, namely, their organizational and activational effects. Sexual differentiation of

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behavior results from organizational (or morphogenic) action of androgens and (or) estrogens within the brain at discrete sensitive periods of development (Feder, 1981; Clarke, 1982; Gorski, 1985). These early effects of gonadal steroids are irreversible, in contrast with the reversible activational actions that steroids have in stimulation of sexual behavior after puberty.

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The semen quality in the buffalo bull depends on the degree of the normality of their testes, epididymes and genital tract (including the accessory sex gland), whereas variations in semen quality are affected by season, temperature, age, health (Saeed, 1988) and hormonal status of animals. Worldwide the number of sperms has decreased in men in last 4 to 5 decades, the sperm quality affected by estrogenic and nonestrogenic chemical compounds (Fisch and Golden, 2003). The sexual activity is changed by various receptors of steroids and quantitative difference of various hormone concentrations. Since beginning the estrogen and testosterone are considered as female and male hormones, respectively. However, both of these hormones are produced in either sex (Hess *et al.,* 2003). Estrogen is also believed to have role in the strength of libido in men; its exposure decreases the risk and delays the onset and progression of important disease like alzheimer's and schizophrenia (Luis, 2000). Estrogen is produced in sizable quantities in the testis, in very high concentrations in the semen of several species has been found, as well as in the brain (Roselli *et al.* 1997), although, the concentration of estrogens in peripheral blood is typically low in the male and ranges from 2-180 pg/ml depending upon the species (Bujan *et al.,* 1993; Eiler and Graves, 1997).

Diethylstilbestrol (DES) is a synthetic estrogenic compound that is several times more potent than estrogen and hundreds to thousands folds more potent than compounds of exogenous environmental estrogens. It is biologically plausible that in utero exposure to estrogen can affect sperm counts; in studies with mice DES is associated with decreased sperm production and abnormal sperm morphology in adult offspring (McLachlan, 1981).

In males (Rat, Rabbit etc.) estrogen is present in low concentrations in blood, but very high in semen, and in rete testis fluids the concentration of this hormone is 250 pg ml^{-1} (Ganjam, 1976; Free and Jaffe, 1979; Toney and Danzo, 1988), this concentration is even higher than serum estradiol level in the female (Smith *et al.,* 1975). Estrogen receptors are present in various reproductive tissue of male (Cooke *et al.,* 1991, West 1990), but the significant role of estrogen in male reproduction is still unclear. The development of a knockout mouse discovery that illustrated the role of estrogen in male, it was shown that functional estrogen receptor is necessary for fertility in both sexes (Lubahn *et al.,* 1993).

The production of estrogens and response by reproductive tissue of male support the concept of role of this important hormone in the male (O'Donnell *et al.,* 2001). It has been reported in various studies that the exogenous environmental estrogens may affect the male sexual behavior (Solano *et al.,* 2000) and fertility in animal models (Akingbemi, 2001). Diethylstilbestrol, a synthetic estrogenic compound has been used in various species to determine the role of estrogen

excess on the reproductive tract (Visser *et al.,* 1998). Research on the impact of exogenous estrogen on sexual behavior in other mammals like bull is required to compare this species with others, and therefore the present study was conducted to evaluate the impact of exogenous estrogen on sexual behavior of Kundhi buffalo bull with the objectives to determine the impact of DES on sexual activity and libido.

MATERIALS AND METHODS

Selection of animals

Four Kundhi buffalo bulls of aged 5-6 years kept under good managemental conditions were chosen for the study, the animals were divided into two groups, in group-1 having three numbers of bulls were treated as experimental animals and these animals were also used as internal control for group-11; prior to treatment, the semen ejaculations from these three bulls were collected and fourth bull was kept as an untreated external control in group-11. The intensity of sexual activity, number of false mounting completed at least in 15 minutes and thrusted vigorously with both hind feet moving forward synchronously, degree of intromission calculated on the basis insertion of male organ in AV with little or more assistance and erection were observed in individual bull during and before the semen collection was recorded as low, medium and full. All these parameters were collected for period of 12 weeks with frequency of 4 ejaculates in a week; the collected qualitative data was converted in numeric before analysis.

Management

The bulls were fed routine ration throughout the study period, and vaccinated against most common diseases as per vaccination schedule, and regularly checked for any abnormality or infection. Health history of the animal from available records was also reviewed. Before semen collection the animals were trained for couple of weeks, during the entire study period the bulls were not allowed for natural service.

Treatment

Bulls of experimental group were given an adult animal dose of 20 mg Diethylstilbestrol injected intramuscularly twice in week to each experimental bull as per recommendation by manufacturer (Star Laboratories Pvt. Ltd. Pakistan). Control animal was treated with same quantity of normal saline.

Statistical analyses

Sexual activity and erection was given numerical numbers according the strength before the analysis of data, the low intensity was given number 1, in case of moderate it was 2 and number 3 was given when animals were observed having higher activity and erection.

Analysis of variance (ANOVA) was applied to analyze the data using the Graph Pad Insat3, Prism 6. The data are presented as average of week wise mean values with standard deviation.

RESULTS

Number of false mountings

The average number of false mounting recorded in control animals was 1.73±0.57, whereas in experimental animals treated with DES was 1.9±0.60, in experimental animals the highest number of false mounting was recorded in bull-3 followed by bull-2 and bull-1 and lowest in group of bulls kept as internal control, although this difference was non-significant.

Table 1. Mean number of false mountings of entire week during the collection of semen from Kundhi Buffalo bulls.

Week	Control	Bull-1	Bull-2	Bull-3
1	2	2	2	2
2	1	2	1	2
3	2	1	2	1
4	2	2	2	2
5	2	2	2	2
6	2	1	1	3
7	1	3	2	3
8	2	1	3	2
9	1	2	2	1
10	3	2	2	1
11	2	2	2	3
12	2	2	2	2
AVG	1.73	-	1.90	-
STD	0.57	-	0.60	-

P = 0.9042

Sexual activity

The sexual activity monitored during the process of semen collection was moderate i.e. 1.58±0.62 in control animal whereas in treatment group it was 1.77±0.87, in bull-1 it was calculated as 2 and 1.66 in bull-2 and bull-3, the difference among all animals was non-significant.

Intromission

Average quantified intromission observed during semen collection was 1.62±0.54 and 1.52±0.51 in control and experimental bulls respectively, the highest values was found in internal control animals and lowest in bull-3 of experimental animals, however, there was non-significant difference between both groups and between animals.

Table 2. Sexual activity observed during the collection of sample from Kundhi Buffalo bulls.

Week	Control	Bull-1	Bull-2	Bull-3
1	2	2	2	1
2	2	2	1	1
3	1	3	2	2
4	1	1	3	1
5	3	2	3	1
6	1	2	1	3
7	2	3	1	3
8	1	3	3	1
9	2	1	1	2
10	1	3	1	1
11	1	1	1	3
12	1	1	1	1
AVG	1.58	-	1.77	-
STD	0.62	-	0.87	-

P=0.6721

Table 3. Intromission of Kundhi buffalo bulls during copulation.

Week	Control	Bull-1	Bull-2	Bull-3
1	1	2	2	1
2	1	1	1	1
3	2	1	1	2
4	2	2	2	1
5	1	1	1	2
6	2	2	2	1
7	2	1	1	1
8	1	2	2	2
9	1	2	2	1
10	2	2	1	1
11	2	1	2	2
12	2	2	2	2
AVG	1.62	-	1.52	-
STD	0.54	-	0.51	-

P=0.8931

Erection

The quantified erection observed during semen collection as described in materials and methods was 1.58±0.54 in control animal and 1.55±0.50 in experimental animals; the lowest intromission was observed in bull-3 and highest in bull-1 of experimental group, the difference in erection between both groups and all animals was non-significant.

Table 4. Erection of the male genital organ during sexual excitement.

Week	Control	Bull-1	Bull-2	Bull-3
1	1	2	2	1
2	1	2	1	1
3	2	1	1	2
4	2	2	2	1
5	1	2	1	2
6	2	1	2	1
7	2	2	1	1
8	1	2	2	2
9	1	2	2	1
10	2	1	1	1
11	2	1	2	2
12	1	2	2	2
AVG	1.58	-	1.55	-
STD	0.54	--	0.50	-

P=0.7822

DISCUSSION

The study was conducted to evaluate the impact of DES on sexual parameters of Kundhi buffalo. The penile erection in experimental and control animals was normal which may be due to the fact that estrogen did not affect the erection and suppressed the level of testosterone and estrogen, as penile erection derives more from neural changes and the neural elements regulating erection, similar studies on the role of androgen agonists and antagonists on the restoration of penile erection have been investigated in rats (Gary *et al.,* 1980). The variation in results may be due to species specific and possibility of lack of several estrogen receptors in the testes and suppression of testosterone.

In the current study the sexual behavior observed during and before semen collection was lower in control animals whereas, in experimental animals it was slightly higher. The male sexual behavior in buffaloes is similar but less intense than that in cattle bull (Janudeen and Hafez, 1992), circulating estrogens also inhibit enzymes involved in testosterone synthesis and may directly affect testosterone production and consequently may decrease the production of estrogens. In men estrogen decline the testosterone levels which is in turn affect the spontaneous erections (during and sexual activity (Kwan, 1983). Free testosterone was more suppressed by the estrogen treatment than total testosterone, as a result of elevated sex hormone binding globulin (SHBG) levels and consequent binding of testosterone. Because severe decreases in sexual function occurred when free testosterone level was reduced, but total testosterone was not below the normal range (Davidson *et al.,* 1982). In this study the administrated exogenous estrogen might be utilized by the estrogen receptors present in brain and other various tissue of the body which result in the slight stimulation of sexual excitement.

Since estrogen circulating in the blood can negatively feed-back to reduce circulating levels of FSH and LH. In men, the major hormone involved in LH feedback is estradiol, not testosterone. Semen production is correlated with sexual behavior in only the fair and poor categories of buffalo bulls. Because estrogen auto regulates its own receptors, a constant dose of estrogen will not likely produce a constant serum concentration; these results are in agreement with Wibowo *et al.* (2011) who suggest that estrogen effectiveness could be optimized if administered cyclically. Sexual activity, erection is inhibited by estrogen (Carosa, 2004). The amount of estrogen is very high in semen and various testcilar tissue of mnay species (Claus *et al.,* 1992; van der Molan *et al.* 1981; Payne *et al*. 1976; Levallet *et al.* 1998) the difference in sexual behavior in this study may be due to lower estrogen level (our unpublished data on semen) and the receptors present in brain which may have important role for sexual behavior.

CONCLUSION

It was concluded that the exogenous estrogens has no or little impact on the sexual activity and libido of buffalo bull, the numerical score of sexual behavior, erection, false mounting and intromission in experimental animals after DES treatment were not improved significantly. These results indicate that unlike other species the estrogen receptors may not be present in the testes and/or epididymis of Kundhi buffalo bulls. Further studies at molecular level like immunohisto chemistry, relative gene expression of estrogen receptors at mRNA level are required to discover the presence and the specific cellular localization estrogen receptor in the testes of buffalo bulls are required, additionally the studies on examination of semen may be an important clue to discover the effect of estrogen on semen quality particularly of sperm motility of Kundhi buffalo bull.

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