Journal of Animal and Poultry Production

Journal homepage: www.japp.mans.edu.eg
Available online at: www.jappmu.journals.ekb.eg

Effect of Hormonal Administration on Reproductive Development of Holstein Bull Calves and Concomitant Changes in Semen Characteristics and Seminal Plasma

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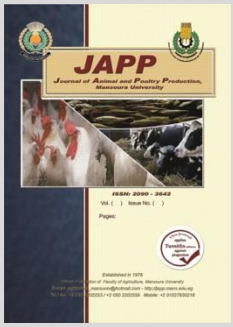

ABSTRACT

This study aimed to determine semen quality and seminal biochemicals at 1st ejaculate, puberty, and maturity of Holstein bulls administrated with GnRH, hCG and testosterone. Holstein male calves (n=16, 8 mo old) were divided into four groups, 4 in each. During 10-weeks interval, each animal was bi-weekly i.m injected with saline (2 ml), 2 ml GnRH, 1500 IU hCG, and 2 ml testosterone. Results indicated the earliest ages to 1st ejaculation, puberty, and maturity by hCG. Ejaculate volume was higher (P<0.05) by GnRH. Sperm motility and livability increased (P<0.05) by GnRH. Seminal total proteins (TP) and albumin (AL) decreased (P<0.05) by GnRH and hCG. Globulin (GL) and fructose decreased (P<0.05) by treatments in the 1st ejaculate. At puberty and maturity, TP, AL, and fructose increased (P<0.05) by treatments. GL increased (P<0.05) by GnRH and hCG. AL:GL increased by GnRH and testosterone at puberty, and by testosterone at maturity. Triglycerides in 1st ejaculation increased (P<0.05) by GnRH. At puberty, HDL, LDL, cholesterol, and triglycerides in GnRH; LDL, cholesterol, and triglycerides in hCG; and triglycerides in testosterone increased (P<0.05). while HDL in hCG and testosterone decreased (P<0.05). At maturity, LDL and cholesterol in hCG, and triglycerides in GnRH and hCG increased (P<0.05), while HDL decreased (P<0.05) by treatments.This study concluded that the treatment of Holstein male calves with GnRH or hCG (once/two weeks) for 10 weeks prior to puberty (8 months of age) leads to early sexual puberty-maturity ages and semen with good quality and chemical composition.

Keywords: GnRH, hCG, Testosterone, Puberty, bull

INTRODUCTION

Puberty is the age when a bull first produces an ejaculum containing 50 × 10^6 sperm of which >10% are progressively motile (Wolf *et al*., 1965). This definition provides a specific endpoint, provided attempts at seminal collection are made with sufficient frequency. From the age of 18 months, the bull was successfully used for semen production in an artificial insemination center. However, at late age, the bull showed a progressive decrease in semen characteristics that involved sperm concentration and volume, motility and sperm abnormalities that resulted in an unsuitable quality of semen for cryopreservation (Contri *et al*., 2012). If the animals reach puberty and sexual maturity at early age, more benefit would be obtained from their productive life. Thus, remarkable physiological changes and/or phenotypic features associated with occurrence of puberty constitute helpful tools for the selection of breeding males (Shaaeldin *et al*., 2019).

The gonadotropins are actively involved in male reproductive functions (Bearden and Fauquay, 1980) and stimulate the secretion of testosterone hormone required for spermatogenesis and sperm transport. Gonadotropin-releasing hormone (GnRH) produced in the hypothalamus stimulates the anterior pituitary gland to produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Whirledge and Cidlowski, 2010), which in turn stimulate Leydig and Sertoli cells in the testes, respectively (Plant and Marshall, 2001; Depenbusch et al. 2002; Shiraishi et al., 2012). Testosterone and FSH are important endocrine factors that act in testes to regulate normal spermatogenesis (Walker, 2010). GnRH and human chorionic gonadotropin (hCG) are considered important hormones used in reproduction and valuable tools for testing whether the function of the male reproductive endocrine system is optimal (Gábor et al., 1998, Parlevliet et al., 2001). Testosterone is the most important of androgens and is secreted by the testicular interstitial cells of Leydig. Testosterone and its by-products, androsterone and dehydrosterone are bound to plasma proteins and are rapidly used by target organs and degraded by the liver and kidneys. Testosterone and related hormones are responsible for male secondary sex characteristics, body conformation, muscular development and libido. They are also responsible for the growth and development of secondary sex glands of the males, as well as maintenance of the viability of the spermatozoa; they stimulate penile growth and separation from the prepuce (Shaaeldin et al., 2019). Testosterone is important for maintaining spermatogenesis, and the absence of testosterone or the androgen receptor results in sterility (Walker, 2011). It has been demonstrated that hCG induces a rapid increase in testosterone biosynthesis in the Leydig cells of the testes (Shiraishi et al., 2012).

Screening of the semen at initial stages can provide insight into the fertilizing capacity of the spermatozoa (Januskauskas and Zilinskas, 2002). A few studies on the effect of GnRH on semen characteristics in cow bulls are

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DOI: 10.21608/jappmu.2020.161227

available (Sajjad et al., 2007; Contri et al., 2012). The time necessary to improve semen quality in the treated bull being 8–9 weeks corresponds to the time spermatogenesis takes (Barth and Oko, 1989).

Most studies were subjected to study age of puberty and maturity without characterization of semen produced at each reproductive stage. Therefore, this study aimed to determine physical semen characteristics and biochemical composition of the seminal plasma at different reproductive stages of Holstein bulls administrated with exogenous hormones (GnRH, hCG and Testosterone).

MATERIALS AND METHODS

Animals used in this study were taken from herd of Sakha Animal Production Research Station and semen processing was carried out at Semen Processing laboratory of International Livestock Management Training Center, Sakha (ILMTC), belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from September 2019 to September 2020.

Animals:

A total of 16 Holstein male calves, at pre-pubertal stage, having live body weight (LBW) from 150 to180 kg and 8 months of age. Animals were subjected to clinical examination of the reproductive tract to exclude any abnormalities of the reproductive organs.

Animals were housed in a semi-open shaded yard and fed based on the recommendation of Animal Production Research Institute (APRI). During winter and spring months, animals were fed on berseem (Trifolium alexandrinum), concentrate feed mixture (CFM) and rice straw (RS), while during summer and autumn months, animals were fed the same CFM, berseem hay (BH), corn silage and RS to cover their nutritional requirements according to their LBW.

Experimental design:

At the beginning of experiment, the experimental animals were assigned randomly into four groups, 4 in each. During an interval of 10 weeks, animals in all groups were i.m. injected bi-weekly with different hormones (6 injections/animal). Animals in the 1st group (G1) were kept as control and injected with normal saline (2 ml/animal) as placebo treatment. In the second group (G2), each animal was injected with 2 ml GnRH (1 ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin; Receptal® VET.; MSD Animal Health). In third group (G3), animals were injected with 1500 IU hCG (EPIFASI; 5000 I.U. of Human Chorionic Gonadotrophin and 10 mg of lactose/1 amp). In the fourth group (G4), animals were injected with 2 ml of testosterone (CIDOTESTON; 250 mg Testosterone Enanthate/1 ml; 1 amp).

Reproductive stages:

After treatment, all animals were observed to determine the pre-pubertal stage (mounting, erection, and 1st ejaculation), the stage of puberty (ejaculates with sperm cell concentration (SCC) of 50 x 10^6 and motility higher than 10%), and stage of sexual maturity when ejaculate reached to SCC of 1000 x 10^6/ml).

Semen collection and evaluation:

Semen was taken from four animals in each group twice/week from 8 months of age up to maturity stage. Semen was collected by means of an artificial vagina and using an intact bull as teaser.

During the experimental period, semen volume was recorded directly from graduated test tubes, then semen pH value was measured with a digital pH meter. The mass activity of spermatozoa was recorded immediately after collection by examining a drop of semen on a warm slide at 10x magnification using a light microscope with attached stage warmer (37°C). The sperm livability and abnormality were determined on a pre-warmed slide by mixing a drop of semen with a drop of eosin-nigrosin stain. A total of 100 sperm cells was counted for each semen sample in five microscopic field. A sample of semen (0.02 ml) was diluted with physiological solution (NaCl 0.9%) and placed in a macro-cuvette to determined sperm cell concentration (SCC) by using spectrophotometer (SDM 1 photometer).

Sperm counts:

Sperm outputs per ejaculate, in terms of total (TSO), motile (MSO), live (LSO), normal (NSO), and functional (FSO) sperm were calculated as the following equations:

TSO/ejaculate = Ejaculate volume (ml) x SCC (x10^6/ml)

MSO/ejaculate = TSO/ejaculate x sperm motility (%)

LSO/ejaculate = TSO/ejaculate x live sperm (%)

NSO = (TSO/ejaculate x (sperm abnormality-100)/100

FSO= [TSO/ejaculate x sperm motility (%) x sperm livability (%) x sperm normality (%)]

Seminal plasma characteristics:

On day of semen collection, the seminal plasma was collected for biochemical analyses. Semen was centrifuged at 3000 rpm for 8 min, then seminal plasma was isolated. Biochemical parameters, including fructose, total proteins, albumin, globulin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides concentrations were determined in the seminal plasma using commercial kits and spectrophotometer.

Statistical analysis:

All data were statistically analyzed using computer program of SAS (2004). Data of ages, response intervals, and seminal plasma analyses were statistically analyzed by one way-ANOVA. All semen characteristics were analyzed by factorial-ANOVA to test the difference between treatment groups and stages. Differences among means were tested using Range Multiple test of Duncan (1955). All percentage values were transformed to arcsine values pre the statistical analysis, then the true values were tabulated.

RESULTS AND DISCUSSION

Results

Age at puberty and maturity:

According to the effect of treatment with exogenous GnRH, hCG and Testosterone on age at puberty and maturity of bulls in the experimental groups (Table 1), hCG treatment showed the earliest ages to produce the 1st ejaculation, and age at puberty and maturity as compared to control (G1), but did not differ significantly from that in G2, and was earlier significantly than in G4, which showed the latest ages. Despite of these differences, hCG treatment showed significantly ($P<0.05$) the shortest interval from 1st ejaculate to maturity, and from puberty to maturity, but did not differ significantly from that in other treatment groups.

Table 1. Age of bulls and intervals between reproductive stages in the experimental groups.

Item	G1 (control)	G2 (GnRH)	G3 (hCG)	G4 (Testosterone)
Age (day)				
At first ejaculate	454.75±19.67^{ab}	413.75±6.26^{ab}	406.00±7.26^{b}	478.33±46.03^{a}
At pubertal ejaculate	463.50±20.37	420.75±6.98	413.25±6.38	484.33±46.64
Age at maturate ejaculate	508.00±22.50^{ab}	448.50±15.95^{b}	436.75±3.33^{b}	523.00±43.02^{a}
Interval (day):				
From first to pubertal ejaculate	8.75±1.31	7.00±1.00	7.25±1.44	6.00±1.00
From first to maturate ejaculate	53.25±4.31^{a}	34.75±9.86^{ab}	30.75±3.97^{b}	44.67±5.33^{ab}
From pubertal to maturate ejaculate	44.50±3.80^{a}	27.75±9.47^{ab}	23.50±3.07^{b}	38.67±6.33^{ab}

^{a,b}: Significant group differences.

Interval of response to treatments:

The intervals from the last injection with exogenous hormones to 1st ejaculation, puberty, and maturity were shorter in G2 and G3 than in G1 (control), and was the longest in G4, but the differences were significant (P<0.05) only between G3 and G4, being the shortest in G3. These results indicated the highest response to treatment with hCG in comparing with other treatments (Table 2).

Table 2. Intervals from treatment to 1st ejaculate, puberty and maturity in the experimental groups.

Item	G1 (control)	G2 (GnRH)	G3 (hCG)	G4 (Testosterone)
Interval (day) from end of treatment to:				
First ejaculate	139.75±19.67^{ab}	98.75±6.26^{ab}	91.00±7.26^{b}	163.33±46.03^{a}
Pubertal ejaculate	148..50±20.37	105.75±6.98	98.25±6.38	169.33±46.64
Maturate ejaculate	193.00±22.50^{ab}	133.50±15.95^{b}	121.75±3.33^{b}	208.00±43.02^{a}

^{a,b}: Significant group differences.

Semen physical parameters:

Volume, pH value, and sperm concentration:

Data in Table 3 showed that ejaculate volume (EV), pH value, and sperm cell concentration (SCC) at each reproductive stage, as well as the overall mean of SCC showed insignificant differences among the experimental groups. However, the overall mean of EV was significantly (P<0.05) higher in G2 than in other groups, while pH value was significantly (P<0.05) lower in G3 than G4, but both did not differ significantly from that in other groups.

Table 3. Volume, pH value, and sperm concentration in ejaculates of bulls in the experimental groups at different reproductive stages

Item	G1 (control)	G2 (GnRH)	G3 (hCG)	G4 (Testosterone)
Ejaculate volume (ml):				
First ejaculate	1.75±0.10	2.30±0.58	2.03±0.16	1.83±0.09
Pubertal ejaculate	1.75±0.10	3.38±1.20	2.33±0.27	1.83±0.09
Maturate ejaculate	4.25±0.43	6.75±0.78	5.00±0.54	4.55±1.05
Overall mean	2.58±0.38^{b}	4.14±0.74^{a}	3.12±0.45^{b}	2.74±0.55^{b}
Semen PH value:				
First ejaculate	8.40±0.12	8.32±0.13	8.13±0.20	8.50±0.10
Pubertal ejaculate	8.40±0.12	8.08±0.29	8.13±0.20	8.50±0.10
Maturate ejaculate	7.05±0.06	7.15±0.09	7.03±0.09	7.23±0.03
Overall mean	7.95±0.20^{ab}	7.85±0.18^{ab}	7.76±0.18^{b}	8.08±0.22^{a}
Sperm cell concentration (1x10^{6}):				
First ejaculate	29.50±5.39	37.75±2.78	36.50±5.64	23.67±5.78
Pubertal ejaculate	78.50±7.63	97.25±21.09	87.50±4.84	68.33±9.26
Maturate ejaculate	1580.50±79.70	1437.75±112.82	1498.00±99.24	1518.33±227.48
Overall mean	526.83±218.40	524.25±197.95	540.67±206.39	536.78±254.13

^{a,b}: Significant group differences.

Sperm characteristics:

Results presented in Table 4 revealed that the percentages of motility, livability, and abnormality of spermatozoa at each reproductive stage, or the overall mean of sperm abnormality did not differ significantly among the experimental groups. However, the overall mean of sperm motility and livability significantly (P<0.05) increased to the maximal values in G2 in comparing with other treatment and control.

Table 4. Sperm characteristics in semen of bulls of the experimental groups at different reproductive stages.

Item	G1 (control)	G2 (GnRH)	G3 (hCG)	G4 (Testosterone)
Sperm motility (%):				
First ejaculate	8.75±2.39	25.00±10.61	17.50±5.95	3.33±1.67
Pubertal ejaculate	18.75±5.91	26.25±11.25	28.75±4.27	10.00±2.89
Maturate ejaculate	47.63±10.36	66.25±4.27	66.25±7.73	61.67±13.64
Overall mean	25.04±6.18^{b}	39.17±7.53^{a}	37.50±7.06^{ab}	25.00±10.07^{b}
Sperm livability (%):				
First ejaculate	9.25±2.06	29.00±10.24	16.75±4.19	4.33±2.60
Pubertal ejaculate	20.50±7.50	28.50±12.00	29.75±4.40	13.00±3.79
Maturate ejaculate	51.00±9.29	66.50±4.73	63.75±5.69	61.00±15.10
Overall mean	26.92±6.45^{b}	41.33±7.31^{a}	36.75±6.48^{ab}	26.11±9.92^{b}
Sperm abnormality (%):				
First ejaculate	23.00±1.96	18.50±3.57	21.00±2.04	23.67±2.19
Pubertal ejaculate	20.25±0.85	16.00±3.11	18.25±2.98	19.33±2.85
Maturate ejaculate	6.25±0.48	8.00±0.91	8.50±0.87	7.33±0.88
Overall mean	16.50±2.31	14.17±1.98	15.92±1.97	16.78±2.67

^{a,b}: Significant group differences.

Total sperm output:

Results illustrated in Fig. 1 showed that sperm outputs (total, motile, live, normal and functional) per ejaculate showed slow increment at puberty as compared to the 1st ejaculation, but a remarkable increase was recorded in all sperm outputs at maturity in comparing with ejaculates at puberty. At all reproductive stages, all outputs were the highest in G2, moderate in G3 and G4, and the lowest in G1 (control).

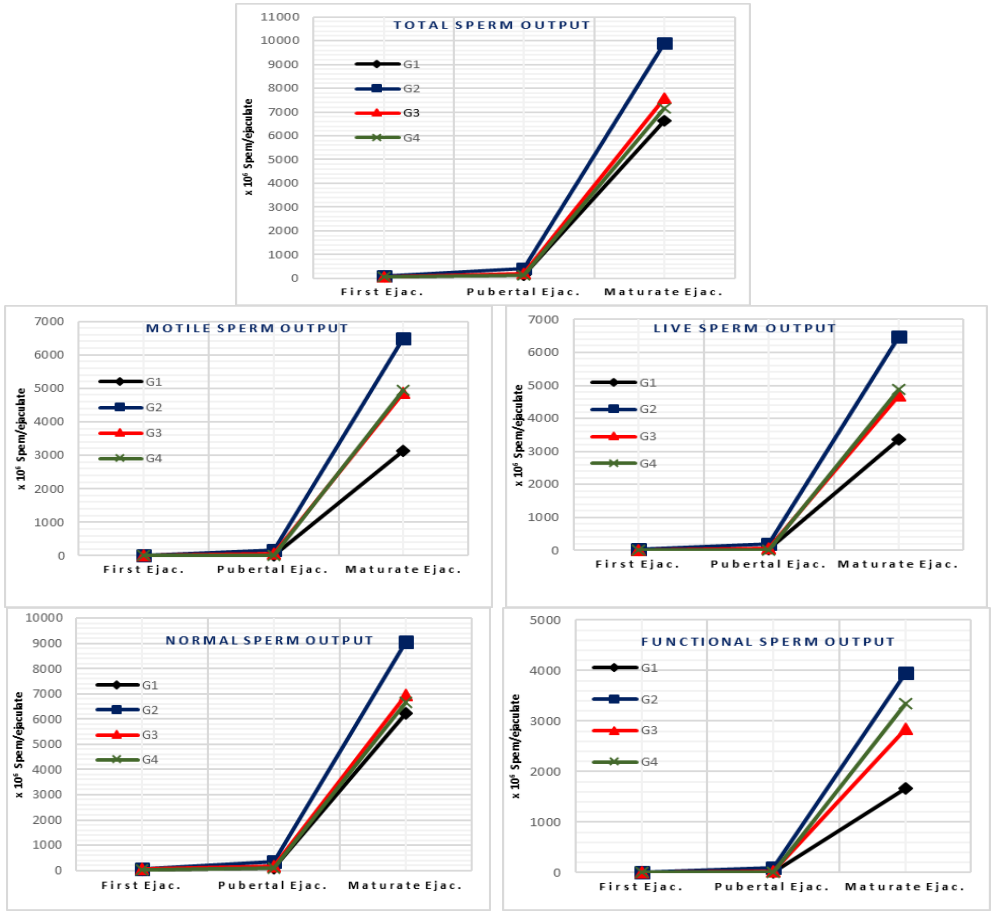

Fig. 1. Changes of total sperm outputs in semen of bulls in the experimental groups at different reproductive stages.

Fructose and total proteins fractions:

The obtained results indicated different trend of changes in all biochemical studied at different reproductive stages (Table 5). Biochemical analysis of total proteins (TP) and albumin (AL) concentrations in the seminal plasma of the 1st ejaculate were significantly (P<0.05) decreased in G2 and G3, but globulin (GL) and fructose concentrations significantly (P<0.05) reduced in all treatment groups as compared to control. These results significantly (P<0.05) reflected the highest albumin/globulin ratio only in G4.

Table 5. Concentration of fructose and total proteins fraction in the seminal plasma of the experimental groups at different reproductive stages.

Item	G1 (control)	G2 (GnRH)	G3 (hCG)	G4 (Testosterone)
Fructose (mmol/l):				
First ejaculate	353.25±19.12^{a}	147.25±16.42^{c}	164.25±10.50^{cb}	207.67±1.76^{b}
Pubertal ejaculate	208.00±4.64^{d}	384.00±6.28^{a}	291.25±5.11^{b}	268.00±14.22^{c}
Maturate ejaculate	249.75±2.29^{c}	360.00±0.41^{a}	207.75±2.43^{d}	307.67±2.85^{b}
Albumin (mmol/l)				
First ejaculate	0.96±0.04^{a}	0.54±0.08^{b}	0.67±0.07^{b}	0.96±0.04^{a}
Pubertal ejaculate	0.67±0.18^{d}	2.30±0.04^{a}	0.95±0.06^{c}	1.63±0.18^{b}
Maturate ejaculate	0.71±0.05^{c}	2.00±0.04^{a}	0.74±0.03^{c}	1.23±0.09^{b}
Globulin (mmol/l)				
First ejaculate	0.28±0.01^{a}	0.17±0.04^{b}	0.15±0.02^{b}	0.16±0.02^{b}
Pubertal ejaculate	0.22±0.01^{b}	0.41±0.04^{a}	0.24±0.01^{b}	0.22±0.01^{b}
Maturate ejaculate	0.16±0.02^{bc}	0.32±0.01^{a}	0.17±0.01^{b}	0.13±0.01^{c}
Total proteins (mmol/l)				
First ejaculate	1.24±0.04^{a}	0.71±0.12^{b}	0.82±0.08^{b}	1.12±0.04^{a}
Pubertal ejaculate	0.89±0.06^{d}	2.71±0.08^{a}	1.19±0.06^{c}	1.85±0.17^{b}
Maturate ejaculate	0.86±0.07^{c}	2.33±0.05^{a}	0.91±0.03^{c}	1.36±0.09^{b}
Albumin: Globulin ratio				
First ejaculate	3.53±0.23^{b}	3.56±0.43^{b}	4.50±0.21^{b}	6.36±0.99^{a}
Pubertal ejaculate	3.13±0.33^{c}	5.76±0.50^{b}	3.96±0.29^{c}	7.48±1.04^{a}
Maturate ejaculate	4.53±0.18^{b}	6.16±0.11^{b}	4.33±0.23^{b}	10.03±1.39^{a}

^{a,b,c}: **Significant group differences.**

At puberty and maturity, concentration of TP, AL, and fructose was significantly (P<0.05) higher in all treatment groups than control group, being the highest in G2. However, GL concentration significantly (P<0.05) increased in G2 and G3, while AL:GL ratio significantly increased in G2 and G4 at puberty, and in G4 at maturity in comparing with control group (Table 5).

Lipid profile:

Results showed that only triglycerides concentration in the 1st ejaculation significantly (P<0.05) increased in G2 as compared to control group. At puberty, HDL, LDL, cholesterol, and triglycerides concentrations in G2, LDL, cholesterol, and triglycerides concentration in G3, and triglycerides in G4 significantly (P<0.05) increased, while only HDL concentration in G3 and G4 significantly (P<0.05) decreased in comparing with control group. At maturity, LDL and cholesterol in G3, and triglycerides concentration in G2 and G3 significantly (P<0.05) increased, while all treatments significantly (P<0.05) decreased HDL concentration compared with control (Table 6).

Table 6. Lipids profile in the seminal plasma of the experimental groups at different reproductive stages.

Item	G1 (control)	G2 (GnRH)	G3 (hCG)	G4 (Testosterone)
HDL (mmol/l)				
First ejaculate	0.60±0.10	0.74±0.08	0.58±0.10	0.79±0.11
Pubertal ejaculate	0.94±0.06^{b}	1.15±0.03^{a}	0.79±0.08^{bc}	0.62±0.04^{c}
Maturate ejaculate	0.98±0.05^{a}	0.61±0.02^{bc}	0.76±0.10^{b}	0.52±0.06^{c}
LDL (mmol/l)				
First ejaculate	15.49±1.05^{ab}	13.10±1.00^{b}	16.57±0.65^{a}	16.63±0.93^{a}
Pubertal ejaculate	11.84±0.44^{b}	17.57±0.65^{a}	18.30±0.80^{a}	14.33±1.37^{b}
Maturate ejaculate	16.04±1.88^{b}	16.07±0.36^{b}	27.07±0.54^{a}	15.24±0.58^{b}
Total cholesterol (mmol/l)				
First ejaculate	16.50±1.04^{ab}	14.50±0.96^{b}	17.75±0.75^{a}	18.00±1.00^{a}
Pubertal ejaculate	13.00±0.41^{b}	19.25±0.63^{a}	19.50±0.87^{a}	15.33±1.33^{b}
Maturate ejaculate	17.25±1.93^{b}	17.00±0.41^{b}	28.25±0.48^{a}	16.00±0.58^{b}
Triglycerides (mmol/l)				
First ejaculate	2.04±0.27^{b}	3.31±0.44^{a}	3.01±0.37^{ab}	2.90±0.20^{ab}
Pubertal ejaculate	1.13±0.02^{c}	2.65±0.03^{a}	2.08±0.10^{b}	1.93±0.09^{b}
Maturate ejaculate	1.18±0.05^{c}	1.63±0.10^{b}	2.13±0.09^{a}	1.20±0.10^{c}

^{a,b,c}: Significant group differences.

Discussion

Age at puberty varies depending upon several factors such as genotype, nutrition and season, ranging from 36 to 49 weeks in Holstein bulls (Killian and Amann, 1972). The present study aimed to evaluate physical semen characteristics and biochemical composition of the seminal plasma at different reproductive stages of Holstein bulls administrated with GnRH, hCG and Testosterone. In the present study pre-pubertal male calves at 8 mo of age were used to determined age at puberty and maturity. The obtained results showed that bulls injected with HCG and GnRH reached to puberty and maturity earlier than those treated with testosterone and controls. Also, both treatments showed the highest response to treatments. The hCG stimulates interstitial cells to produce androgens in particular testosterone which affects the sexual glands and sperm production through creation of the best conditions for spermatogenesis (Ranghraz Tavakoli *et al*., 2018). Also. hCG induces a rapid increase in testosterone biosynthesis in the Leydig cells of the testes (Shiraishi *et al*., 2012). Although the using exogenous GnRH to increase the rate of development had been unsuccessful, because calves were treated too late in development (Amann, 1983), the earlier reproductive stages of bulls treated with GnRH as compared to untreated bulls in our study may be attributed to GnRH in our study started at early age (8 mo). In this context, exogenous GnRH increases LH pulse frequency at 4-6 weeks of age in Hereford bull calves (Chandolia *et al*., 1997). The GnRH treatment of beef calves, twice daily, from 4 to 8 weeks of ag, prior to the normal endogenous early increase in LH secretion, hastened the onset of sexual maturity, increased the testicular tissues, and reduced age at puberty (Madgwick *et al*., 2008). The negative effects of testosterone treatment on delaying all reproductive stages as compared to control bulls in our study may be attributed to that testosterone administration may suppress the episodic release of GnRH via the hypothalamus, delayed the onset of puberty in bull calves (Schanbacher, 1982; Godfrey *et al*., 1989).

Screening of the semen at initial stages can provide insight into the fertilizing capacity of the spermatozoa (Januskauskas and Zilinskas, 2002). Treatment of cow bulls with GnRH affected semen characteristics (El-Azab *et al*., 1996; Gábor *et al*., 1998). In our study, bulls treated with GnRH showed better semen characteristics than other groups. This is in agreement with Contri *et al*. (2012), who reported that the continuous administration of a low dose of a GnRH analog resulted in recovery of the function of the testis, in corroboration with the improvement of semen characteristics to normal within 9 weeks. As a consequence, the time necessary to improve semen quality in the treated bull being 8–9 weeks corresponds to the time spermatogenesis takes as reported in the literature (Barth and Oko, 1989). GnRH treatment resulted in decreased sperm mid-piece and tail abnormalities in Nili-Ravi buffalo bulls (Sajjad *et al*., 2007). The decrease in semen characteristics of bulls treated with testosterone in or study may be due to the decrease in LH secretion that occurs in these species leads to reduced testosterone production and regression of the testes, with chronic exposure to agonist eventually inducing azoospermia. Bulls treated with buserelin had normal mean plasma LH concentrations and elevated testosterone (Rechenberg *et al*., 1986). Concentration of ejaculates has been considered vital for the production of maximum number of the semen straw in order to get maximum number of doses for artificial insemination. In agreement with the present results, GnRH treatment of beef calves, twice daily, from 4 to 8 weeks of ag, prior to the normal endogenous

early increased LH secretion, hastened semen ejaculate outputs (Madgwick *et al*., 2008). Generally, the duration of spermatogenesis in bulls was estimated at 61 days (Barth and Oko, 1989). This finding may explain the development in all semen characteristics from the 1st ejaculation to puberty or from puberty to mature ejaculation. In our study, treatment of GnRH and hCG reduced the interval between both reproductive stages.

Seminal plasma constituents include ions, energy substrates (mainly fructose in case of bull sperm), organic compounds, peptides, and proteins. The protein constituent of seminal plasma is vital in efficient fertilization, as it influences spermatozoa plasma membrane stability, motility, capacitation, and sperm egg interaction (Juyena and Stelletta, 2012). The chemical analysis of the seminal plasma indicated pronounced changes in plasma biochemicals occurred as affected by hormonal treatments and even by advancing the reproductive. Treatment with GnRH significantly ($P<0.05$) improved fructose, total proteins and their fraction, and lipid profile in the seminal plasma of bulls at puberty and maturity. The biochemical evaluation of the seminal plasma is an important criterion for assessing fertility and diagnosing male reproductive disorders (Massanyi *et al*., 2004 a, b). To better understand the potential role of seminal plasma interaction with spermatozoa at ejaculation, a parallel could be made with epididymal maturation. Epididymal sperm maturation consists in a progressive acquisition of mobility and zona pellucida binding as sperm transit along the epididymis (Cornwall, 2009; Dacheux and Dacheux, 2014; Sullivan and Mieusset, 2016). Considering the sperm membrane proteome, epididymal maturation and interaction with seminal plasma proteins can be considered as a continuous process of differentiation. The sugar composition of seminal plasma (fructose) has also been correlated with fertility, mainly due to its importance to spermatozoa energy production. Fructose and glucose are essential for ATP production and motility of spermatozoa (Jobim *et al*., 2004; Assumpcao *et al*., 2005). A key feature in the function of spermatozoa is the lipid composition of seminal plasma and sperm membrane. Cholesterol has a special relevance since it is the most abundant lipid in the spermatozoa of all mammalian species (Cross, 1998). Cholesterol and triglyceride levels were significantly higher in normozoospermic bulls in comparison to oligoasthenozoospermic bulls.

CONCLUSION

This study concluded that the treatment of Holstein male calves with GnRH or hCG (once bi-weekly) for 10 weeks prior to puberty (8 months of age) leads to early sexual puberty-maturity ages and semen with good quality and chemical composition. This may help to obtain bulls at early maturity age and longevity for artificial insemination. Also, this may provide basic knowledge to early hasten the sexual development in bull calves, and may be helpful in the selection of sires at pre-maturity stage.

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تأثير المعاملة الهرمونية على التطور التناسلى لعجول الهولشتين و التغيرات المصاحبة فى خصائص السائل المنوى و البلازما المنوية

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هدفت الدراسة إلى تقييم جودة السائل المنوى و التحليل الكيميائى لبلازما السائل المنوى فى مرحلة أول قذفة منوية ، البلوغ و النضج الجنسى لعجول الهولشتين المحقونة بهرمونات (GnRH ، hCG و التستوستيرون) . تم استخدام 16 عجل هولشتين فى مرحلة ما قبل البلوغ عند عمر 8 شهور . قسمت هذه الحيوانات الى أربعة مجموعات ، 4 حيوانات فى كل مجموعة. حقن كل حيوان كل اسبوعين لمدة 10 اسابيع بـ 2مل محلول ملح ، 2 مل GnRH ، 1500 وحدة دولية hCG و 2 مل تستوستيرون. و تبين من النتائج أن المعاملة بـ hCG أدت الى إنتاج الحيوانات أول قذفة منوية ، الوصول الى البلوغ و النضج فى عمر مبكر. كان متوسط حجم القذفة المنوية كبير معنويا ($P<0.05$) فى المعاملة بـ GnRH ، النسبة المئوية للحيوية و الحيوانات المنوية الحية للقذفة المنوية ارتفعت معنويا ($P<0.05$) فى المعاملة بـ GnRH. تركيزات البروتين الكلى و الألبيومين فى البلازما المنوية للقذفة الأولى انخفضت معنويا ($P<0.05$) فى معاملتى GnRH و hCG، لكن تركيزات الجلوبيولين والفركتوز انخفضت معنويا ($P<0.05$) فى جميع المعاملات في مرحلة أول قذفة منوية. فى مرحلتى البلوغ والنضج الجنسى ، تركيزات البروتين الكلى و الألبيومين و الفركتوز ارتفعت معنويا ($P<0.05$) فى المعاملات. بينما تركيز الجلوبيولين ارتفع معنويا ($P<0.05$) فى معاملتى GnRH و hCG . معدل الالبيومين الى الجلوبيولين ارتفع معنويا ($P<0.05$) فى معاملتى GnRH و التستوستيرون فى مرحلة البلوغ ، و فى معاملة التستوستيرون فى مرحلة النضج الجنسى. فى مرحلة أول قذفة منوية ارتفع تركيز الدهون الثلاثية معنويا ($P<0.05$) فى معاملة الـ GnRH . فى مرحلة البلوغ الجنسى ، تركيزات HDL ، LDL ، الكولستيرول و الدهون الثلاثية فى معاملة الـ GnRH ، و تركيزات LDL ، الكوليسترول و الدهون الثلاثية فى معاملة الـ hCG ، و الدهون الثلاثية فى معاملة التستوستيرون زادت معنويا ($P<0.05$). وجد انخفاض معنوى ($P<0.05$) فى تركيز HDL فى معاملتى الـ hCG و التستوستيرون. فى مرحلة النضج الجنسى ، كانت تركيزات LDL و الكولستيرول فى معاملة الـ hCG ، و الدهون الثلاثية فى معاملتى GnRH و hCG مرتفعة معنويا ($P<0.05$) ، بينما انخفضت تركيزات HDL معنويا ($P<0.05$) فى جميع المعاملات. وتستنتج هذه الدراسة أن حقن عجول الهولشتين بهرمون GnRH او hCG فى مرحلة ما قبل البلوغ (حقنة كل أسبوعين) لمدة 10 أسابيع عند عمر 8 شهور أدى إلى البلوغ والنضج الجنسى المبكر و تحسين خصائص السائل المنوى و مكونات القذفة المنوية.