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EFFECT OF rbST ADMINISTRATION ON PUBERTAL AGE AND SOME BLOOD PARAMETERS IN FRIESIAN HEIFERS

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SUMMARY

To investigate the effect of rbST treatment in relation to some hormones (IGF-I and GH) and metabolites (glucose and urea-N) on the initiation of early puberty in Friesian heifers, a total of 16 Friesian heifers having 187.63±2.56 kg live body weight and 8.23±0.59 months of age was divided into two similar groups, 8 animals each. Animals in the first group (G1) were subcutaneously (s.c.) injected with saline (0.9% Nacl) while, animals in the second group (G2) were s.c. injected with 250 mg rbST 14 d-interval for five times pre-puberty. Feeding system and management were the same for both groups. Blood samples were collected to determine concentration of P4, GH, IGF-I, glucose and urea-N in blood plasma throughout the experimental period from 8.2 mo of age up to puberty. Results show insignificant differences in average P4 concentration during 3 wk pre-puberty, P4 peak pre-puberty and interval from P4 peak to puberty. The interval from 1st rbST injection to puberty was earlier (83.67 vs. 136.0 d, P<0.05) and LBW was lighter (254.6 vs. 279.1 kg) in G2 than in G1. Concentration of P4 at puberty was not affected by treatment. Average age at puberty was 342 and 391d in G2 and G1, respectively. Overall concentration of P4 during the experimental period was nearly similar in G1 and G2, being 0.460 and 0.472 ng/ml, respectively. Concentration of IGF-I and GH was higher (P<0.05) in G2 than in G1 as overall mean or pre- and at puberty. This increase was about 20 and 19% for IGF-I and 61 and 41% for GH pre- and at puberty, respectively. The differences in IGF-I and GH between pre- and at puberty for each group were not significant. Average concentration of IGF-I showed sharp increase post the 1st rbST injection by about 24.5%. Overall concentration of glucose during the experimental period increased (P<0.05) in rbST group (85.9 mg/dl) as compared to the control group (79.1 mg/dl), although glucose concentration pre- and at puberty was not affected significantly by rbST treatment. Concentration of urea-N was lower (P<0.001) in G2 than in G1 as overall mean (27.5 vs. 32.2 mg/dl). Also, concentration of urea-N reduced (P<0.05) in G2 compared with G1 pre- and at puberty by 15 and 19%, respectively. Pre-pubertal urea-N concentration in heifers showed marked reduction by increasing number of injections, in particular post-1st injection. The strongest positive correlation was between concentration of GH and IGF-I (r= 0.695, P<0.001).

In conclusion, rbST treatment at a level of 250 mg at 14- day interval for five successive times pre-puberty is strongly in relation to concentration of GH and IGF-I and in less extend to glucose and urea-N concentration to induce precocious puberty in Friesian heifers

Keywords: rbST, pubertal age, blood parameters, Friesian heifers

INTRODUCTION

Administration of exogenous somatotropin (ST) influences the somatotropic axis, improves body weight gain and feed efficiency (Moseley *et al*., 1992 and Rausch *et al*., 2002), and increases the number and size of ovarian follicles (Lucy *et al*., 1994) and growth of corpus luteum and progesterone (P4) production (Gallo and Block, 1991). The somatotropic axis is closely associated with pubertal development in heifers. Chandrashekar *et al*. (2004) suggested a vital role for GH and IGF-I in the control of pituitary and gonadal functions in animals and humans.

In this respect, Lucy (2000) found that ST and the IGF-I are important hormones for ovarian follicular growth. Receptors of ST and IGF-I are present in follicular cells. In addition, the granulosa and theca cells of the follicle are sites of IGF-I and IGF-II synthesis, respectively. Somatotropin increases ovarian IGF-I synthesis. The IGF is important for follicular growth because both IGF-I and IGF-II are synergistic with gonadotropins for growth and differentiation of ovarian follicles.

The possible involvement of growth hormone (GH) in the regulation of ovarian follicular growth and development in mammals has been suggested by several observations. Therefore, circulating concentrations of GH increase rapidly during pubertal ages (Ojeda and Jameson, 1977). The effects of ST and IGF-I on the onset of puberty of heifers (195 kg) were studied by Simpson *et al.* (1991). They proposed that ST and IGF-I are important metabolic mediators involved in the initiation of puberty in heifers.

To identify metabolic hormones that serve as metabolic cues for onset of puberty, Jones *et al.* (1991) determined changes in GH and IGF-I before puberty in heifers. Frequency of GH release was greater at day -40 to -17 from puberty. Concentrations of IGF-I (measured every 2 wk) increased linearly (P<0.07) from day -56 to 0 day from puberty. Mejia *et al.* (1999) suggested that enhanced pre-pubertal IGF-I levels in conjunction with increased pre-pubertal LH levels and pubertal LH pulse amplitude might be involved in the accelerated somatic maturation and in puberty advancement observed in heifers. Somatotropin increased circulating concentration of glucose an effect that may be attributed to increased hepatic gluconeogenesis and reduced uptake of glucose by adipose tissue (Hall *et al*., 1994).

Aim of the present study was to investigate the effect of rbST treatment in relation to some hormones (IGF-I and GH) and metabolites (glucose and urea-N) on the initiation of early puberty in Friesian heifers.

MATERIALS AND METHODS

A total of 16 Friesian heifers having 187.6±2.56 kg live body weight and aged 8.2±0.59 months was divided into two similar groups, 8 animals in each**.** Animals in the first group (G1) were injected subcutaneously (s.c.) with 2 ml sterilized physiological saline (0.9% Nacl) at the same time; animals in the second group (G2) were s.c. injected with 250 mg rbST (Somatech® of Elilly) at intervals of 14 days. The injection started at the 252 days (8.2 mo.) for successive five times pre-puberty. All heifers were free of any diseases with healthy appearance and they were housed in separated two groups under semi-open sheds, partially roofed with asbestos.

Feeding system and management

Heifers in both groups were fed on equal amounts of diet containing the CFM, rice straw and fresh berseem (during winter season) or berseem hay (during summer season) according to the recommendation of the NRC (2001) allowances for growing dairy heifers based on live body weight. Diets were fed to both groups twice daily at eight am. and three pm., while fresh water was available all daytime.

Representative monthly samples of feedstuffs were chemically analyzed for CP, CF, EE, NFE and ash on DM basis according to the official methods of the A.O.A.C (1995). Chemical composition of CFM, rice straw, fresh berseem and berseem hay used in feeding heifers in both groups is shown in Table (1).

Determenation of puberty

At the beginning of the ninth month of age, vasoectomized male was introduced to heifers of each group for 20 min. three times daily at 6 and 12 am. and 6 pm. to detect heifers exhibiting the 1st oestrous activity. The onset of the 1st oestrus was used as an indicator for the onset of puberty. In addition, ovulatory activity of heifers at puberty was also indicated as P4 concentration exceeded one ng/ml for two consecutive sampling days **(**Jones *et al.*, 1991 and Simpson *et al.*, 1991) in blood plasma collected from heifers during the experimental period. Interval from rbST treatment to heifers reaching puberty was recorded.

Table 1. Chemical analysis of different feed stuffs (on dry matter basis) used in feeding heifers

Item	**Chemical composition (%)**			
	CFM	**Rice straw**	**Fresh berseem**	**Berseem hay**
Dry matter, DM	**90.22**	**89.24**	**15.26**	**88.23**
Organic matter, OM	**89.76**	**83.22**	**86.15**	**88.58**
Crude protein, CP	**16.04**	**1.59**	**14.71**	**14.41**
Crude fiber, CF	**10.96**	**37.21**	**24.9**	**24.67**
ether extract, EE	**4.91**	**1.47**	**2.90**	**6.04**
Nitrogen free extract, NFE	**56.38**	**42.85**	**43.64**	**43.16**
Ash	**10.24**	**16.78**	**13.85**	**11.42**

Experimental procedures

Blood sampling

Blood samples were collected from the jugular vein of each animal in both groups starting before rbST treatment and 3-4 day-interval throughout the experimental period (170 days). Blood samples were centrifuged at 3000 rpm for 10 minutes to separate blood plasma and stored at -20 ^{0}C until analysis.

Concentration of P4 was 3-4 day-interval determined at the beginning of the experiment up to puberty incidence. Throughout the whole experimental period, concentrations of GH and IGF-I were weekly determined and concentration of glucose and urea-N were monthly determined in plasma.

Hormonal assay

Direct radioimmunoassay technique (RIA) was performed for determination of plasma P4 concentration using antibody-coated tubes kit (Diagnosis systems, laboratories Texas, USA) according to the procedure outlined by the manufacture. The standard curve of progesterone concentration ranged from 0 to 2.4 ng/ml. The intra- and inter-assay coefficients of variation were 5.4 and 9.1%, respectively.

Plasma GH and IGF-I were analyzed by radioimmunoassay. Iodation of GH and IGF-I and analyses were done according to the procedures previously described by Van Wyk (1983) and Daughaday and Rotwein (1989), respectively. The GH used for analysis was radioimmunoassay-grade. The inter- and intra-assay coefficients of variation for the GH analysis were 11.8 and 5.1%, respectively. Radioimmunoassay grade IGF-I was purchased according to manufactory (Thebarton, SA, Australia). The inter- and intra-assay coefficients of variation for the IGF-I analysis were 10.6 and 7.7%, respectively.

Blood biochemical analysis

Concentration of glucose and urea-N in blood plasma were determined according to Trinder (1969) and Patton and Crouch (1977), respectively, using commercial kits (Diagnostic System Laboratories, Inc USA) and spectrophotometer.

Statistical analysis

The obtained data were statistically analyzed according to Snedecor and Cochran (1982) and correlation coefficients were determined using SAS (2004) according to the following model: $Y_{ij} = U + A_i + e_{ij}$ where: Y_{ij}= observed values, U= overall mean, A_i= treatment and e_{ij}= random error.

RESULTS AND DISCUSSION

Progesterone profile

Results presented in Table (2) show insignificant group differences in average P4 at 3 wk prior to puberty, P4 peak determined prior to puberty and interval from P4 peak to puberty incidence. However, the interval from first rbST injection to puberty was significantly (P<0.05) earlier in G2 than in G1, reflecting significantly (P<0.05) earlier incidence of puberty and insignificantly lighter weight of heifers in G2 than in G1. However, P4 concentration at puberty was not affected by treatment. It is of interest to note that the observed heavier weight of heifers at puberty in G1 was attributed to incidence of earlier puberty in G2 than in G1. Results illustrated in Figure (1) revealed that plasma P4 concentrations were almost higher in treated than in control group at all sampling pre-pubertal days. Average age of all heifers to attain puberty (progesterone greater than 1 ng/ml for two consecutive sampling days) was 342 and 391days (11.4 and 13.0 mo of age) in treated and control group, respectively, being lower (P<0.05) in heifers injected with rbST than in those control group by 49 days (1.6 mo). It is of interest to note that overall concentration of P4 during the experimental period was nearly similar in both of treated and control group, being 0.472 and 0.460 ng/ml, respectively. These findings proposed that rbST is important to induce the initiation of puberty in heifers. Similar results were obtained by Simpson *et al.* (1991) in heifers injected with growth hormone-releasing factor (GRF) and human serum albumin (HAS). Also, Aboul-Wafa (2009) found marked increase in P4 concentration in blood serum of ewes treated with rbST as compared to the controls.

In addition to its known metabolic effects, GH has been shown to have direct effects on function of ovarian granulose cells in rats and pigs (Hsu and Hammond, 1987 and Adashi *et al*., 1989). Some studies have shown that GH treatment *in vivo* can stimulate growth of small follicles in pre-pubertal pigs (Spicer *et al*., 1990) and increase ovulation rates in cyclic gilts (Kirkwood *et al*., 1988). The mechanism for the increase in follicular growth and ovulation rate is unclear.

Concentration of IGF-I and GH

Data in Table (3) show that overall concentration of IGF-I and GH during the experimental period was significantly (P<0.001) higher in treated than in control groups. Also, concentration of IGF-I and GH pre- and at puberty was significantly (P<0.05) higher in treated than in control group. This increase was about 20 and 19% for IGF-I and 61 and 41% for GH pre- and at puberty, respectively. It is of interest to note that concentration of IGF-I increased at puberty as compared to pre-pubertal values while, GH concentration showed different trend in both groups. However, the differences in IGF-I and GH between pre- and at puberty for each group were not significant.

Results illustrated in Figure (2) reveal that plasma IGF-I concentrations were almost higher in treated than in control group at all sampling pre-pubertal days. Average concentration of IGF-I showed sharp increase post the 1st injection in treated heifers, resulting in large differences from that in control group by about 24.5%. These differences continued to be present after the subsequent injections, indicating higher pre-pubertal IGF-I concentrations in treated than in control group as affected by rbST injection.

It is worthy noting that GH concentration showed the same trend of change as IGF-I as affected by rbST injection, but the differences between treated and control group were reduced by increasing number of rbST injections from the 1st one (Figure 3).

In similarity with the present study, Stelwagen *et al*. (1993) and Slaba *et al*. (1994) found a 3-7 fold-increase in plasma rbST concentrations during the first three post-injection hours in cows treated with rbST. In the next 8 days, the rbST concentration in rbST treated cows was significantly higher than the controls. Plasma concentration of IGF-I increased nearly 2 folds as early as 24 h following rbST treatment and then continued to increase by 48 h post 48 h injection (4 times higher than controls). From 48 h after rbST treatment, IGF-I concentrations remained at a plateau till day 11, then decreased slowly but still remained higher on day 14 than those in controls.

Administration of exogenous bovine GH stimulates growth in growing cattle, including pre-pubertal heifers (Sandles and Peel, 1987; McShane *et al*., 1989 and Vestergaard *et al*., 1993). Growth hormone exerts some of its action via IGF-I, and, consistent with this, the concentration of IGF-I in blood is increased by GH treatment (Crooker *et al*., 1990). The biological effects of IGF-I are modulated by IGF binding proteins (IGFBP), which have been characterized by legend blotting in calves (Skaar *et al*., 1994).

The present results indicated the role of GH and IGF-I that serve as metabolic cues for onset of puberty. The somatotropic axis is closely associated with pubertal development in heifers. Therefore, Chandrashekar *et al*. (2004) suggested a vital role for GH and IGF-I in the control of pituitary and gonadal functions in animals and humans. In this respect, Schams *et al*. (1991) observed an increase of plasma rbST at 7 d after treatment of cows with recombinant Methionyl rbST (500 mg/14 d), and then decreased at 14 d (P<0.01). Also, Jones *et al.* (1991) determined changes in GH, IGF-I and LH before puberty in heifers. They found that frequency of GH release was greater at day -40 than at day -17 of puberty in Angus heifers. In Braford and Charolais heifers, frequency of GH release was greater at day -17 than at day -40 of puberty. Concentrations of IGF-I (measured every 2 wk) increased linearly (P<0.07) rom day -56 to 0 day from puberty in Angus but not in other breeds. In addition, enhanced pre-pubertal IGF-I levels in conjunction with increased pre-pubertal LH levels and pubertal LH pulse amplitude might be involved in the accelerated somatic maturation and in puberty advancement observed in heifers. In this line, Mejia *et al.* (1999) reported that serum IGF-I level increased from birth to 22 wk of age and then reached a plateau. In agreement with the present results, Hodate *et al*. (1991) found that plasma IGF-I concentrations were significantly higher in the rbST treated than in the control heifers at pre-pubertal stage. Similar trend was reported in the rbST treated cows (Hodate *et al*., 1991). Also, treatment with rbST significantly increased IGF-I concentration by 36.7% (121.2 and 77.6 ng/dl for rbST and control group, respectively) as reported by Gallo and Block (1990). Moreover, Tripp *et al.* (1998) found that in heifers and steers, serum ST and IGF-I concentrations increased (P<0.05) by ST administration.

Table 2. Average P4 concentration (ng/ml) during pre-pubertal stage and at puberty, and P4 concentration, age (day) and live body weight (kg) at puberty of Friesian heifers in control (G1) and treated (G2) groups

Item	G1	G2
During pre-pubertal stage:		
Average P4 during 3 wk prior to puberty	0.64±0.03	0.58±0.03
P4 peak prior to puberty	0.85±0.10	0.71±0.10
Interval from P4 peak to puberty (day)	14.0±1.86	11.5±1.86
Interval from first injection to puberty (day)	136.0±3.46[a]	83.67±3.45[b]
At puberty:		
P4 concentration	0.36±0.04	0.41±0.04
Age (day)	391.0±3.5[a]	341.7±3.5[b]
Live body weight (kg)	279.1±18.6	254.6±12.9
Overall mean of P4 during the experimental period	0.46±0.03	0.47±0.04

a and b: Means having different superscripts within the same row are significantly different at (P<0.05).

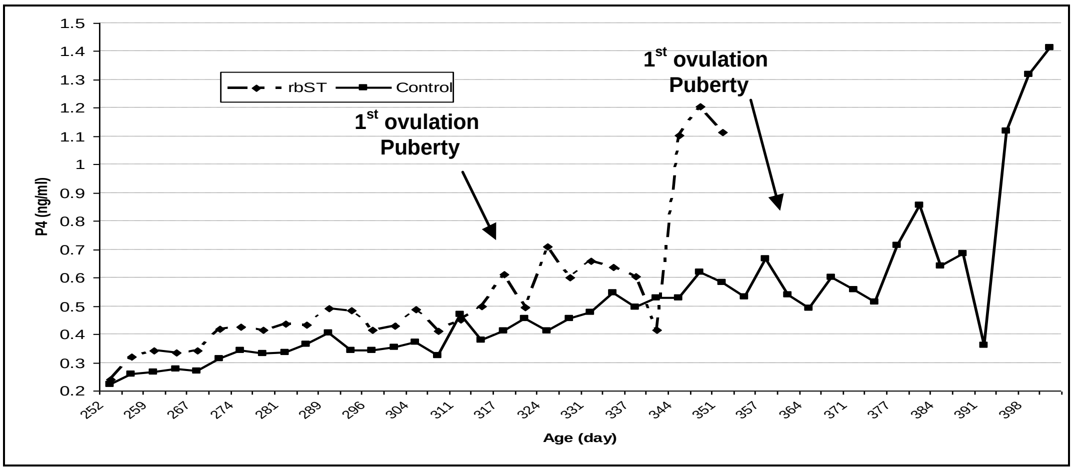

Fig. 1. Plasma P4 concentration in control and treated groups throughout the experimental period measured from rbST injection up to puberty

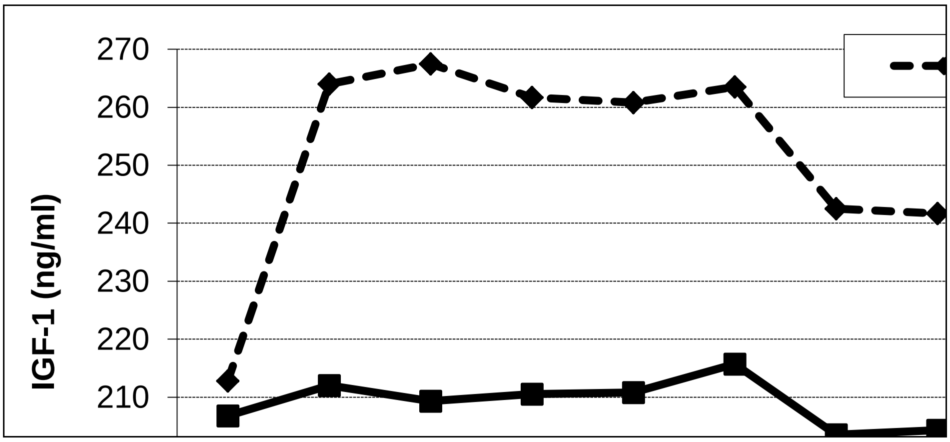

Fig. 2. Change in plasma IGF-I concentration in heifers of control and treated groups throughout the experimental period

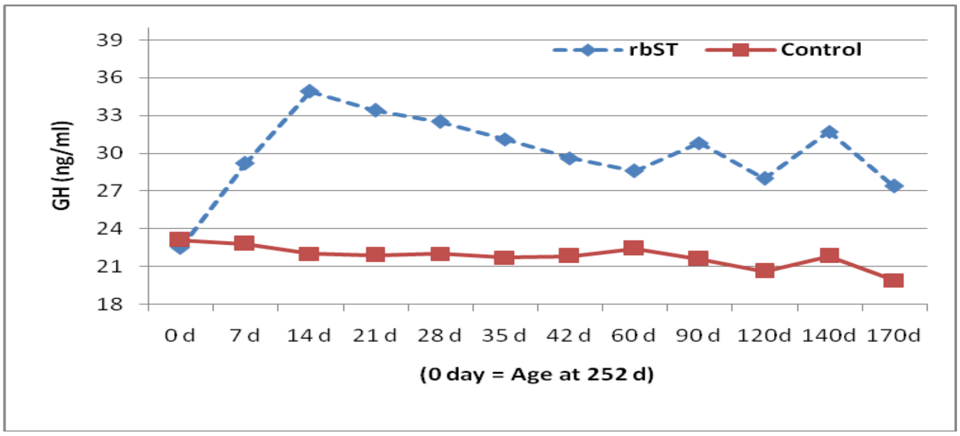

Fig. 3. Change in plasma GH concentration in control and treated groups throughout the experimental period

Table 3. Average concentration of IGF-I and GH (ng/ml) pre- and at puberty in blood plasma of heifers in control (G1) and treated (G2) during the experimental period

Item	Time	G1	G2	Significance
IGF-I	Pre-puberty(1)	189.3±5.4	226.7±5.4	*
	At puberty	204.3±12.0	242.7±12.0	*
	Overall mean	207.25±1.9	248.33±2.4	***
GH	Pre-puberty	19.80±0.57	31.80±0.57	*
	At puberty	21.8±1.5	30.8±1.5	*
	Overall mean	21.80±0.52	29.98±0.32	***

* Significant at $P<0.05$ *** Significant at $P<0.001$. (1): average values of control and treated groups were 8 and 10 wk, respectively.

Concentration of glucose and urea-N

Data in Table (4) show that overall concentration of glucose during the experimental period significantly ($P<0.05$) increased in G2 as compared to G1. Although glucose concentration pre- and at puberty was not affected significantly by rbST treatment, it showed different trends of change pre- and at puberty in each group.

On the other hand, concentration of urea-N was significantly lower in G2 than in G1 as overall ($P<0.001$) or pre and at puberty ($P<0.05$). The reduction in urea-N concentration was about 15 and 19% pre- and at puberty, respectively, and may be attributed to variation in live body weight and metabolic status of heifers during pre- and post-puberty.

As affected by rbST injection, glucose concentration (Figure 4) showed marked increase up to the post 2nd rbst injection in treated group versus marked reduction in control group. However, glucose concentration was higher at puberty in treated than control group, then it increased in control group and still stable in treated group to be similar in both groups at puberty of control group. Such trend suggested incidence of puberty in control group when glucose level reached the same level in treated group. Similar trend of change in glucose level was reported by Gallo and Block (1990), who found that rbst-treatment, resulted in sharp increase in glucose level post-rbst-injection of Friesian cows.

In accordance with the present results, Stevens *et al*. (1980) reported that glucose values in lactating Holstein cows were 65.2, 68.1, 65.1, and 68.6 at rbST levels of 0, 10.3, 20.6 and 41.2 mg/dl, while pre-treatment value was 57.1. Also, Pocius and Herbein (1986) stated that glucose concentration was not affected in plasma of Holstein-Friesian cows in mid lactation injected with GH (50 IU/day) for 11 consecutive days. Furthermore, Morbeck *et al*. (1991) reported that glucose was not influenced by dose of rbST.

In contrast, Molento *et al*. (2002) found that rbST induced insulin peripheral resistance and increased liver gluconeogenesis, or both. Also, Hall *et al*. (1994) stated that ST increased circulating concentration of glucose, an effect that may be attributed to increased hepatic gluconeogenesis and reduced uptake of glucose by adipose tissue.

Concerning the results illustrated in Figure (5), plasma urea-N concentrations were almost lower in treated than in control group at all sampling days pre- and post puberty. It is of interest to note that pre-pubertal urea-N concentration in heifers showed marked reduction by increasing number of injections, in particular post-1st injection, then urea-N concentration showed slight rise post-puberty. This reduction in urea-N concentration in treated heifers may be a reflection of decreased amino acid degradation and utilization of these amino acids for increased milk protein synthesis (Marcek *et al*., 1989), suggesting that treated animals utilized protein more effectively (Whitaker *et al*. 1989 and Hodate *et al*. 1991).

In agreement with the present results, treatment with rbST (640 mg rbST/14d) significantly decreased plasma urea-N concentrations in Holstein heifers (Whitaker *et al*. 1989 and Hodate *et al*. 1991). Also, Early *et al*. (1990) reported that concentrations of serum urea was lower ($P<0.05$) in steers (initially 9 mo of age and 231±18 kg) receiving daily injections of rbST (20.6 mg/d) for 112 d. Moreover, Marcek *et al*. (1989) found that blood urea-N concentration decreased in pregnant lactating Holstein cows treated with 430 mg/d of rbST as compared to control cows. On the other point of view, several authors observed insignificant effect of rbST treatment on urea-N in blood of cows (Schams et *al*., 1991) and primiparous Holstein cows (Morbeck *et al*., 1991).

Correlation coefficients between each of all parameters studied are presented in Table (5). Results show that GH showed the strongest positive correlation with concentration of IGF-I and negatively correlated with urea-N. Schams *et al*. (1991) found that concentrations of IGF-I were positively correlated with changes in ST. Such results may indicate the important role of exogenous ST on increasing concentration of IGF-I as a metabolic mediator involved in the initiation of puberty in heifers (Simpson *et al*., 1991).

It is of interest to note that the correlation between concentration was positive between IGF-I and glucose ($r= 0.314$, $P<0.001$) and negative with urea-N (0.314, $P<0.001$). The present results indicated poor positive correlation between P4 concentration and each of IGF-I, GH, and urea-N, while P4 concentration negatively correlated ($P<0.01$) with glucose concentration.

In conclusion, rbST treatment at a level of 250 mg at 14- day interval for five times pre-puberty is strongly in relation to concentration of ST and IGF-I and in less extend to glucose and urea-N concentration to induce precocious puberty in Friesian heifers.

Table 4. Concentration of glucose and urea-N (X±SE) as measured pre- and at puberty in blood plasma of heifers in control (G1) and treated (G2) groups

Item	Time	G1	G2	Significance
Glucose (mg/dl)	Pre-puberty	75.21±3.92	86.23±3.92	NS
	At puberty	81.68±3.25	80.73±3.25	NS
	Overall mean	79.10±1.2	85.86±1.3	*
Urea-N (mg/dl)	Pre-puberty	33.23±1.08	28.33±1.08	*
	At puberty	31.70±1.54	25.53±1.54	*
	Overall mean	27.48±0.86	32.16±0.92	***

NS: Not significant * Significant at $P<0.05$ *** Significant at $P<0.001$

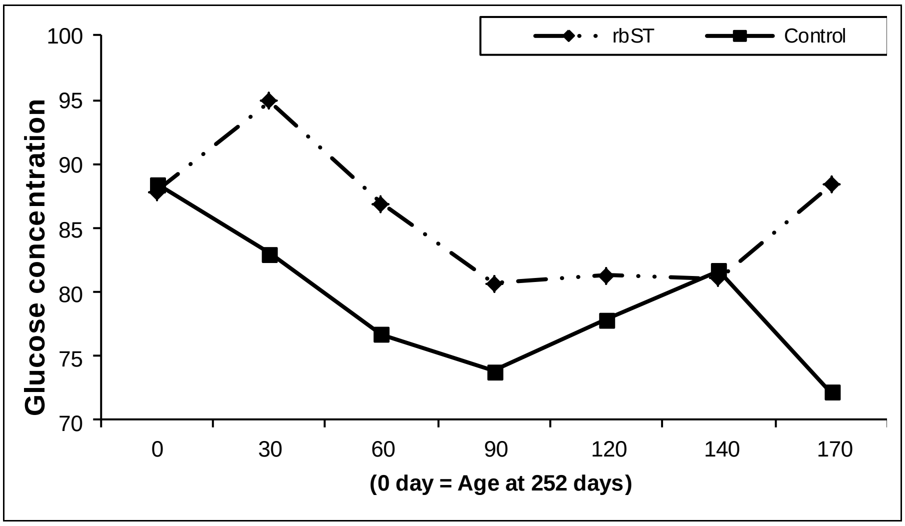

Fig. 4. Plasma glucose concentration before (0 day) and after rbST treatment in control and treated groups

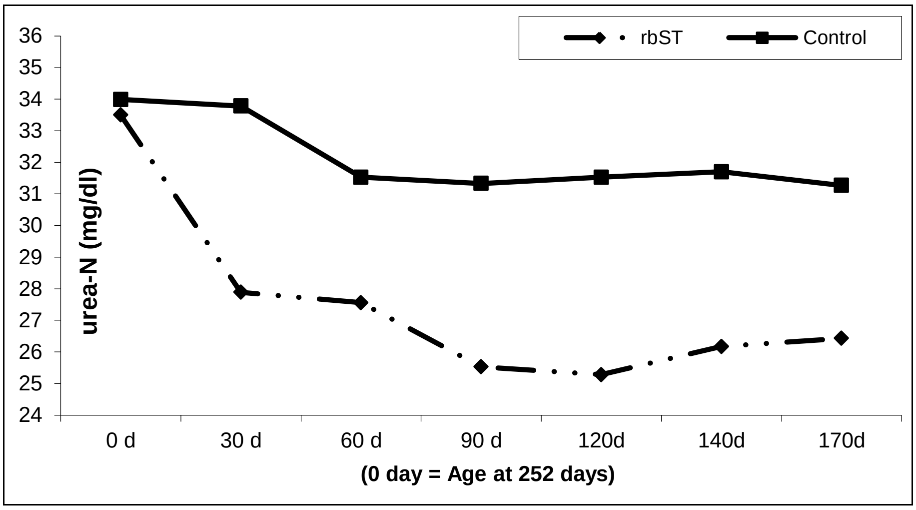

Fig. 5. Change in plasma urea-N concentration in heifers of treated and control groups throughout the experimental period

Table 5. Pearson correlation coefficients between different parameters studied in both treated and control groups

Item	IGF-I	GH	P4	Urea-N
GH	0.69560***			
P4	0.06175^{NS}	0.15344^{NS}		
Urea-N	-0.21743*	-0.18888*	-0.08411^{NS}	
Glucose	0.31459***	0.16080^{NS}	-0.27330**	-0.22963*

NS: Not significant * Significant at P<0.05 ** Significant at P<0.01 ** Significant at P<0.001

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تأثير هرمون النمو المخلق وراثيا علي عمر البلوغ و بعض صفات الدم في عجلات الفريزيان

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استخدم فى هذه الدراسة عدد 16 عجلة فريزيان وزنها 178.63 وعمرها 8.23 شهر من العمر ووضعت فى مجموعتين متشابهتين، 8 حيوانات لكل مجموعة. المجموعة الأولي حقنت تحت الجلد بمحلول فسيولوجي (0.9 % كلوريد الصوديوم)، بينما حيوانات المجموعة الثانية حقنت تحت الجلد ب 250 مليجرام من هرمون النمو (rbST) كل 14 يوم خمس مرات قبل البلوغ. وبدأ الحقن من عمر حوالى 8.23 شهور وحتى 16 شهر من العمر تقريبا. وكان نظام التغذية والرعاية متشابهة في المجموعتين. وجمعت عينات الدم لتقدير تركيز هرمون البروجسترون وهرمون النمو وهرمون IGF-1 الجلوكوز واليوريا في بلازما الدم. وأظهرت النتائج عدم وجود اختلافات معنوية في متوسط تركيز البروجسترون خلال 3 أسابيع قبل البلوغ وأعلي تركيز البروجسترون قبل البلوغ والفترة من أعلي تركيز البروجسترون قبل البلوغ وحتى البلوغ. كانت الفترة الفاصلة من أول حقنة حتى البلوغ أقصر (84 يوم) في المجموعة المعاملة عن الكنترول (136 يوم).أنخفض وزن الجسم الحي في المجموعة المعاملة عن الكنترول (257.6 و279.1 علي الترتيب). كان متوسط عمر البلوغ 342 و391 في في المجموعة المعاملة والكنترول علي الترتيب. لم يختلف تركيز البروجسترون عند البلوغ معنونا في المجموعتين. كان تركيز البروجسترون متساوى تقريبا فى المجموعتين خلال الفتره التجريبيه. ارتفع تركيز هرموني IGF-1 وهرمون النمو قبل وأثناء البلوغ وخلال الفتره التجريبيه في المجموعة المعاملة والكنترول وكانت الزيادة في حدود 20 و19% في IGF-1 و61 و41% قبل وإثناء البلوغ علي الترتيب. ارتفع معنويا تركيز هرمون النمو وIGF-1 فى المعاملة مقارنة بالكنترول فى كل العينات قبل البلوغ. وقد اظهر تركيز IGF-1 زيادة حادة بعد الحقنة الاولى و كانت فى حدود 24.5% بينما كانت الزيادة كبيره فى هرمون النمو بعد الحقنة الثانية. ارتفع تركيز الجلوكوز معنويا خلال الفتره التجريبيه بينما لم يختلف تركيزه معنويا قبل او اثناء البلوغ. انخفض تركيز نيتروجين اليوريا فى الدم معنويا فى المجموعة المعاملة قبل و اثناء البلوغ وكان معدل الأنخفاض 15 و19% على التوالى. اظهر تركيز نيتروجين اليوريا قبل البلوغ انخفاضا ملحوظا بزيادة عدد مرات الحقن وخصوصا بعد الحقنة الاولى. وكان هناك ارتباط موجب (r=0.695) بين تركيز هرمون النمو وIGF-1.

و قد أوضحت نتائج الدراسه المقدمه أن الحقن بهرمون النمو (250 مللجرام) لخمس مرات بفترة بينية 14 يوم قبل البلوغ كان له علاقة قوية لرفع مستوى هرمون النمو و IGF-1 وبدرجه اقل مع مستوى الجلوكوز واليوريا فى التبكير بحدوث البلوغ لعجلات الفريزيان.