Optimizing the Age of Puberty in Buffalo Heifers by Recombinant Bovine Somatotropin (rbST) and/or Gonadotropin Releasing Hormone (GnRH)

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Abstract: The aim of the current study was to investigate the efficiency of recombinant bovine somatotropin hormone (rbST) and/or gonadotropin releasing hormone (GnRH) for induction of puberty in prepubertial buffalo heifers. Twenty eight buffalo heifers, aged 23.3±0.1 months and weighted 370.0±1.6 kg, were randomly allotted into rbST-GnRH treated (G1, n=10); GnRH alone-treated (G2, n=10) and control (G3 n=8). Both follicular populations and largest follicle diameter were detected by transrectal ultrasonography on Days -3 and 0 whereas Day 0 was the Day of GnRH injection. Also, serum IGF-1 and plasma glucose levels were assayed on Day 0.There was an increase (P<0.01) in the serum IGF-1 and plasma glucose levels in G1 on Day 0. Also in G1 heifers, there was a significant increase in the largest follicle diameter on Day 0 (13.6±0.8 m) compared to Day-3 (8.41±1.1 mm). The follicular populations in terms of small and large-sized classes were higher (P<0.05) in G1 compared to G2 and G3 as well. Both puberty induction and conception rates were higher (P<0.05) in G1 compared with other groups.In conclusion, the highest rate of puberty initiation in prepubertial buffalo-heifers is achieved when rbST is injected 3 days prior to GnRH.

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1. Introduction

Delayed puberty, higher age at first calving, tendency for seasonal breeding and silent heat limit reproductive and productive efficiency in buffalo (Qureshi *et al.*, 2002; Sarwar *et al.*, 2009; Terzano *et al.*, 2012 and Ali, 2015). Great variations in the age at attaining puberty in buffalo heifers *vz*: 16-22 to 36-40 months (Borghese et al, 1994); 24-96 months (Kumar *et al.*, 1992); 3-5 years (Nada *et al.*, 2003); 5-39 months (Naqvi and Shami, 1999) and 16-46 months (Perera, 2008) resulted in wide variation and delay in the age of first breeding (Ettema and Santos, 2004) and consequently the age at first calving (Bhatti *et al.*, 2007 and Sarwar *et al.*, 2009). In beef cattle, heifers that calve by 2 years of age have greater lifetime production potential than those which calve at older ages (Patterson *et al.*, 1992 and Ahmedzadeh *et al.*, 2011). Also the tendency of buffalo for seasonal breeding during short day times (Barile, 2005) represents another constrain for heifers whose age of puberty coincides with low breeding season during long day times. Bhatti *et al.* (2007) reported that buffalo-heifers attaining puberty before breeding season are more likely to get bred and have their first calving at lower age than those attaining puberty beyond breeding season and have to wait for their next breeding season.

Mimicking the hormonal changes occurring around puberty may induce sexual maturity in buffalo heifers (Barile, 2005). Insulin-like growth factor-1 was reported to increase during prepubertial period in Bos Taurus heifers (Granger *et al.*, 1989 and Yelich *et al.*, 1995). Treatment of heifers with exogenous growth hormone (GH) was associated with increased circulating concentration of IGF-1 and an earlier onset of puberty (Cooke *et al.*, 2013 and Hall *et al.*, 1994). Insulin like-growth factor-1 stimulated proliferation of granulose cells and acts synergistically with FSH to promote its activity in terms of protein and steroid synthesis in buffalo (Pawshe *et al.*, 1998).

Although the key factor in initiating puberty is the secretion of GnRH/LH in high frequency and amplitude (Ahmedzadeh *et al.*, 2011 and Amstalden *et al.*, 2014), concurrent development of large follicle with high steroidogenic activity is necessary to produce estradiol surge able to induce GnRH/LH surge to induce ovulation (Amstalden *et al.*, 2014 and Samadi *et al.*, 2014). The pituitary gland of 12-months old buffalo-heifers responds to administration of exogenous GnRH but endogenous basal plasma

gonadotrophin concentration are not established until 24 months of age (Singh and Madan, 1998 and Nada *et al.*, 2003).

Not only the effect of growth hormone (GH) on the reproductive processes was restricted to the effects mediated by IGF-s system, oxytocin, steroids and changes in the gonadal receptors to gonadotrophins but its major effects are being through direct effects through specific GH receptors acting through cANP/protein kinase A, protein kinase G and tyrosine kinase-MAP kinase (Sirotkin, 2005).

In view of the previously mentioned beneficial effects of GH in modulating gonadal receptors for gonadotrophins and enhancing growth and steroidogenic activity of the largest follicle, the present study was designed to test the efficiency of rbST and/or GnRH in inducing puberty in buffalo heifers at the prepubertial age.

2. Materials and methods

2. 1. Experimental animals and management:

This study was conducted in Mehallet Mousa buffalo Research Station, during period extending from Nov. 2015 to Feb. 2016. Twenty eight prepubertial Murrah buffaloes (*Bubalous bubalis*) heifers, 23.3±0.1 months in age and 370±1.6 kg in weight, were enrolled in the present study. They were selected on the basis of their reproductive history and absence of corpus luteum in two transrectal ultrasound scans made at 10 days interval.

The heifers were fed according to the recommendation of Animal Production Research Institute (APRI, 1997). The weight of heifers in the current study exceeds critical weight (330 kg) for breeding recorded by Bhatti *et al.* (2007) in buffalo. The feed was formed from pelleted concentrate mixture, berseem hay (*Trifolium Alexandrium*), corn silage and rice straw. They were allowed for free access of water. They were kept indoors throughout the years in semi-sheltered free stalls. They had a normal general healthy conditions and vaccinated against infectious diseases according to recommendations of official local veterinary services authority in Kafr El Sheikh Governorate, Egypt.

2.2. Experimental design:

The experimental animals were randomly assigned into 3 groups, rbST and GnRH treated group (G1, n=10); GnRH alone-treated group (G2, n=10) and control (G3, n=8). Each heifer in G1 received an i.m. injection of 250 mg of sustained formula of rbST (Boost in -250 mg, L.G. Chemical LTS, DaeJeans, South Korea) on Day-3 and 20µg of GnRH agonist (Buserelin, 5 ml Receptal® Intervet International GmbH-Germany, each 1 ml contains Buserelin acetate 4.2 µg equivalent to 4µg buserelin) on Day 0. Each heifer in G2 (GnRH-alone treated) received the same GnRH agonist by the same dose on Day 0. In the control group, each heifer received two i.m. injections of saline on Days -3 and 0.

2. 3. Ultrasound scanning:

The ovaries of the experimental animals were scanned by transrectal examination using an ultrasound scanner (Ultrascan 900, Allianco, Quebec Canada) equipped with a 7.5 MHz linear transducer on Day -3 and 0. The ovarian follicular status in terms of the largest follicle on Days -3 and 0 as well as number of follicles on Day 0 was determined. The number of follicles was recorded in the size categories: ≥ 2 to 7.9, 8-9.9 and ≥ 10 mm.

2.4. Reproductive management:

2.4.1. Heat detection: The heifers were visually observed for estrous detection twice daily (at 6.00 AM and 6.00 PM) by the herd-man for 1 hour at each round check. They were observed for, bellowing, vulvar tumefaction, frequent micturation and vaginal mucous discharge (Perera, 1999). Also standing of heifers to be mounted by the vasoectomized bull maintained with them was the most reliable sign for estrous detection.

2.4.2. Breeding: The heifers were artificially inseminated using proven fertile frozen semen in straws on the basis of AM/PM system i.e. heifers detected in estrous at the evening were inseminated at the morning and vice versa.

2.4.3. Pregnancy diagnosis: The inseminated heifers were observed for estrous return on Day 19-23 post-breeding. The non-return heifers were examined by transrectal scanning of the uterus on Day 30 post-breeding. The conception rate was calculated as the percentage of conceived heifers in relation to total number of inseminated heifers.

2.5. Blood sampling:

From each heifer in all groups on Day 0, two blood samples were collected by jugular vein puncture one into 10 ml heparinized vacutainer tube for obtaining plasma and the other into plain 10 ml vacutainer tube for obtaining serum. Blood samples were centrifuged at 1500 x g for 20 min and the separated plasma and serum were stored in polypropylene tubes at -20^{o}C until determining both plasma glucose and serum IGF-1.

2.6. Assays:

2.6.1. Serum IGF-1 assay: Serum IGF concentrations were measured for all heifers on Day 0 using IGF-1-RIA CT kit, manufactured by DIA source immunoassay S.A. Rue de L'Industrie & B-1400 Nivelles, Belgium. The procedure used was that described in the accompanying Catalogue Nr: KIP1588 and P.I. Number: 1700868/en. The intra-assay coefficient of variation was 9.1 and inter-assay coefficient of variation was 9.0. The sensitivity of the test was 3.4 ng/ml.

2.6.2. Plasma glucose assay: Plasma glucose levels were determined by quantitative enzymatic colorimetric method (Stanbia Enzymatic glucose procedure No. 1075, Stanbia laboratories, Boerne, Texas, USA), according to the procedure described by Trinder (1969).

2.7. Statistical analysis:

The statistical analysis was performed using SAS computer program. The overall means±SE for the size of the largest follicle, follicular population, serum IGF-1 and plasma glucose were computed. The data were analyzed by the least square analysis of variance using the general linear models procedure of SAS (2000). Both estrous response and conception rates within each experimental group were calculated in percentages. The differences in the two parameters among the comparable groups were analyzed by Chi square test.

3. Results

3.1. Ovarian follicular status:

3.1.a. Size of the largest follicle:

The largest follicle diameter showed non-significant variation, prior to treatment, on Day-3 among three groups. On Day 0, the largest follicle diameter showed significant increase in G1 heifers indicating the enhancing effect of rbST on the growth of the largest (dominant) follicle. On the other hand, there was non-significant variation in the largest follicle diameter between Day -3 and 0 in either of G2 or G3 ensuring the enhancing effect of rbST on the follicular growth in G1 (Table, 1).

3.1.b. Follicular populations:

In G1, the rbST treatment on Day -3 significantly (P<0.05) increased total number of follicles on Day 0 compared with other groups (Fig., 1) . The numbers of small and large-sized follicles (8.2±2.0 and 2.1±1.4) in G1 were greater (P<0.05) than either G2 (3.8±1.7 and 0.9±0.3) or G3 (4.1±2.9 and 0.6±0.4) respectively (Fig., 1). However, non-significant variations were recorded in the numbers of medium sized follicles among 3 groups (2.9±2.1for G1, 2.5±1.0 for G2 and 2.6±1.8 for G3, Fig., 1).

3.2. Serum IGF-1 and plasma glucose concentrations on Day 0:

On Day 0, the serum IGF-1 concentration in G1(286.9±10.1 ng/ml) showed significant (P<0.05) increase compared with either G2 (149.8±9.9 ng/ml) or G3 (160.0±12.2 ng/ml) indicating the stimulatory effect of rbST in increasing peripheral serum concentration of IGF-1 (Fig. 2a). Also the plasma glucose level in G1 (78.8±2.1 mg/dL) showed a significant (P<0.05) increase compared to G2 (59.7±2.0 mg/dL) and G3 (61.1±3.3 mg/dL) (Fig., 2b).

3.3. Fertility response:

The percentage of buffalo heifers attaining puberty as indicated by estrous response was the highest in G1 (100%) compared to either G2 (40%) or G3 (25%) (Table 2). It was observed that while none of heifers in either G2 or G3 were detected in estrous within 1-4 days (Day 0 is day of GnRH injection) , 4 heifers were detected in estrous in G1. Although the remaining 6 heifers in G1 were detected in estrous within 5-8 days, only 4 of 10 in G2 and 2 of 8 in G3 were detected in estrous within the same interval.

Regarding conception rate it was noted that there was a significant (P<0.05) increase in G1 compared with either G2 or G3 (Table 2).

Table (1): Means±SE of the largest follicle diameter (mm) in the heifers on Day -3 and 0.

Treatments	Diameter of the largest follicle (mm)	
	Day -3	Day 0
G1	8.4 ± 1.1^{aB}	13.6 ± 0.8^{aA}
G2	8.3 ± 1.2^{aA}	9.1 ± 1.8^{bA}
G3	8.3 ± 1.5^{aA}	9.0 ± 1.6^{bA}

Day -3 = day of rbST injection.
Day 0= day of GnRH injection.

Means within the same column with different small letters are significantly different at P<0.05.

Means within the same rows with different capital letters are significantly different at P<0.05.

Table (2): Estrous response rate (ERR) and conception rate (CR) in different treatment groups.

Group	Total number	ERR in relation to Day 0						Conception rate		
		Days 1-4		Days 5-8		Total		Total No. of responding heifers	Conceived	%
		No	%	No	%	No	%			
G1	10	4/10	40	6/6	100^{a}	10/10	100^{a}	10	7	70^{a}
G2	10	0/10	0	4/10	40^{b}	4/10	40^{b}	4	1	25^{b}
G3	8	0/8	0	2/8	25^{b}	2/8	25^{b}	2	0	0
Total	28	4/28	14.28	12/24	50	16/28	57.14	16	8	50

Means within the same column with different small letters are significantly different at P<0.05.

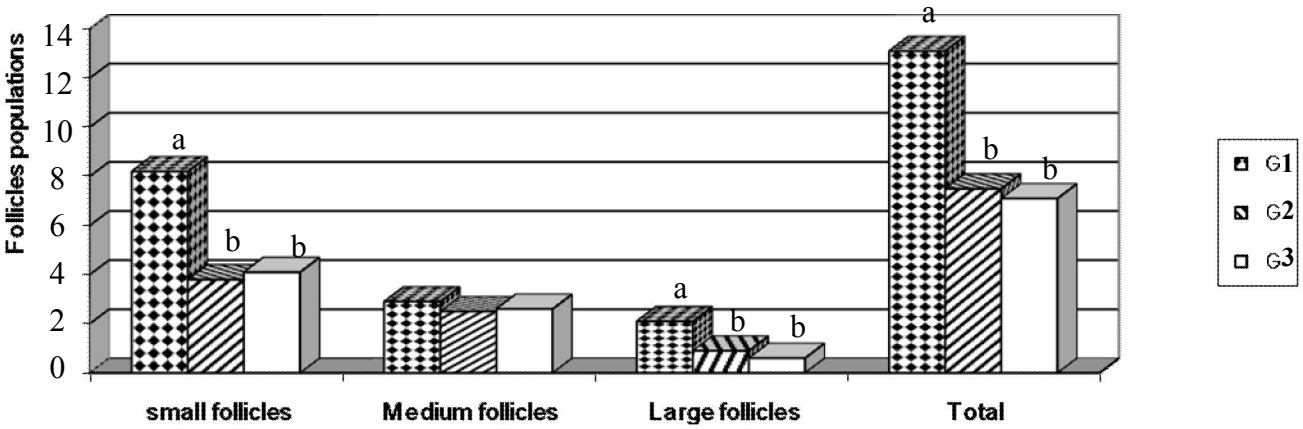

Fig. (1): Number of follicular populations (Mean±SE) in G1, G2 and G3 on day 0. Column with different small letters are significantly different at *P*<0.05.

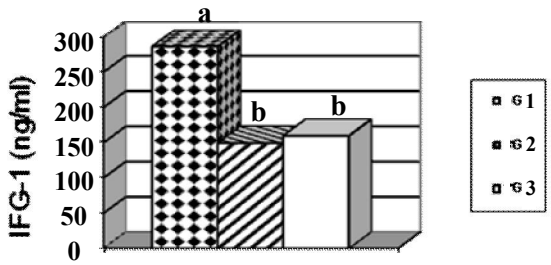

Fig. 2.a

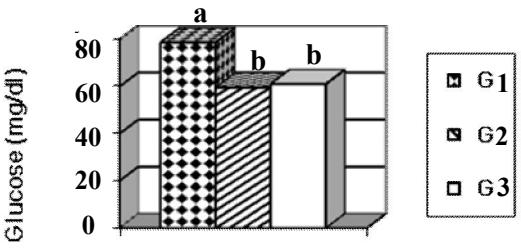

Fig. 2.b

Fig. (2a&b): Mean±SE concentration of serum IGF-1 (Fig. 2a) and plasma glucose (Fig. 2b) on Day 0 in the heifers of the different comparable groups, Column with different small letters are significantly different at P<0.05.

4. Discussion

Optimizing the age of puberty in mature buffalo heifers enables buffalo breeders from having adequate number of breedable heifers especially during breeding season. Thus maximizes number of replacement heifers required for increasing herd size and replacing culled cows which undoubtedly impacted well on the reproductive performance. Also, earlier initiation of puberty in buffalo heifers reduces rearing costs and age at first calving (Gojjam *et al.*, 2011).

The highest estrous response rate in rbST-GnRH-treated buffalo-heifers compared with either GnRH alone-treated or control proved the beneficial effect of rbST as co-treatment with GnRH in inducing puberty in mature buffalo heifers. These results came in accordance with Cooke *et al.* (2013) and Hall *et al.* (1994) in beef heifers. Also, Haldar and Prakash (2006) concluded that the increased plasma GH concentration in response to growth hormone releasing factor (GRF) treatment in buffalo heifers induced earlier puberty.

Several mechanisms have been suggested to explain the enhancing effect of rbST in inducing early puberty in buffalo heifers. Growth hormone has been implicated in regulating the response of reproductive organs to GnRH and gonadotrophins (Sirotkin, 2005). Also, GH has been recorded to increase LH pulse frequency in pigs (Glibertson *et al.*, 1991). However, the stimulatory effect of rbST on the gonadotrophins release may be mediated through increasing plasma glucose level (Bucholtz *et al.*, 1996). Which support this claim in our study is higher plasma glucose level in rbST-treated group compared with other groups. In agreement with higher plasma glucose level in rbST-treated heifers in the current study, Khattab *et al.* (2008) recorded high plasma glucose level following rbST treatment in Egyptian buffaloes.

Not only the enhancing effect of rbST in inducing puberty in buffalo-heifers in the current study was restricted to its effect on the GnRH/gonadotrophins secretion and their receptors formation but the major effect is believed to be mediated through increasing serum concentration of IGF-1 which stimulates growth and steroidogenic activity of follicles (Gong *et al.*, 1997). The significant increase in the serum IGF-1 in the rbST-treated buffalo heifers support the profound effect mediated by it. In accordance with our results, Cooke *et al.* (2013); Gong *et al.* (1991) and Hall *et al.* (1994) recorded an increase in the serum IGF-1 in response to GH treatment in heifers. The increased serum IGF-1 during prepubertial period and at onset of puberty in Bas Taurus heifers (Granger *et al.*, 1989 and Yelich *et al.*, 1995) suggest that it is an important signal for initiation of puberty (Velazquez *et al.* 2008).

Compared with other groups, the concomitant increase in the serum concentration of IGF-1 with the increase in both follicular population and size of the largest follicle in rbST-treated group suggests that the stimulatory effect of rbST on the follicular dynamics is thought to be mediated through the increase in the level of such metabolic hormone which finally impacted on the initiation of puberty in rbST-GnRH treated group. In agreement with the current study, the increase in the number of small (Kirby *et al.*, 1997a) and large follicles (Kirby *et al.*, 1997b) was recorded in rbST-treated dairy cows. In parallelism with the increase in the number of small and large sized follicles, IGF-1 enhanced follicular growth as indicated by significant increase in the diameter of largest follicle. Dupont *et al.* (2014) reported that IGF-1 and insulin have essential role in the final stage of follicle development. Also, Pawshe *et al.* (1998) stated that IGF-1 stimulate proliferation of granulose cells in buffalo and acting synergistically with FSH in promoting steroidogenic activity.

In GnRH alone-treated heifers, although estrous response was detected in 4 out of the treated 10 heifers, only one heifer conceived. This result agree with Singh and Madan (2000) who found that although hormonal treatment with GnRH increased the number of heifers expressing estrous but conception rate was disappointing. In the current study, the reduced size of the largest follicle in GnRH alone-treated heifers may be attributed to low serum IGF-1 which is necessary for final stage of follicle development. Thus the low serum concentration of this hormone may be insufficient to support follicular growth of the largest follicle that underwent atresia (Dupont *et al.*, 2014) with subsequent reduced estrous response.

Also in the same respect, the lower conception rate in GnRH alone treated heifers compared to rbST-GnRH-treated ones may be explained in the light of smaller diameter of the largest follicle on Day 0, (Day of GnRH injection). It was 9.1±1.8 in GnRH-alone treated versus 13.6±0.8 mm in rbST- GnRH-treated heifers.

However, the small sized follicle might result information of small sized CL whose ability to produce P4 was compromised (Roche *et al.*, 2000). On the other hand, ovulation of the larger follicles leads to formation of larger and more functional CL capable of producing P4 suitable for concepts elongation and improved pregnancy rate (Binelli *et al.*, 2014).

In conclusion, the highest rate of puberty initiation in prepubertial buffalo-heifers is achieved when rbST is injected 3 days prior to GnRH.

Conflict of interest statement

All authors declare that they have no relationship with people or organizations that could prejudice or bias the content of this paper.

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References

1. Ahmedzadeh, A., Carnahan, K. and Autran, C., 2011. Understanding puberty and postpartum anestrus. Proceeding of Applied Reproductive Strategies in Beef cattle. September 30-October, 2011, Boise ID.
2. Ali, A., 2015. Seasonal variation of the ovarian activity and pregnancy rate in the Egyptian buffalo cows (*Bubalus bubalis*). Trop. Anim. Health Prod., 47, 815-818.
3. Amstalden, M., Cardoso R. C., Alves B. R. C., Williams G. L., 2014. Hypothalamic neuropeptides and nutritional programming of puberty in heifers. Reproductive Symposium, J. Anim. Sci., 92, 3211-3222.
4. APRI, 1997. Animal Production Research Institute, Animal and poultry Nutrition 1st Ed, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt (In Arabic).
5. Barile, V. L., 2005. Improving reproductive efficiency in female buffaloes. Livestock Prod. Sci., 92, 183-194.
6. Bhatti, S. A., Sarwar M., Khan M. S., Hussain S. M., 2007. Reducing the age of calving through nutritional manipulation in dairy buffaloes and cows: A review. Pakistan Vet. J., 27, 1, 42-47.

7. Binelli, M., Pugliesi G., Hoeck V. V., Sponchiado M., Ramos R. S., Olivera M. L., Franca M. R., D'Alexandri F. L., Mesquita F. S., Membrive C. M. B., 2014. The role of proestrous on fertility and post-ovulatory uterine function in the cow. Anim. Prod.11, 3, 246-253.
8. Borghese, A., Terzano G. M., Barile V. L., Annicchiarico G., Allegrini S., Zicarelli L.,Montemuro N., Pacelli C., Campanile G., Esposit L., Di Palo R., Boni R., Seren E., Parmeggiani A., 1994. Puberty and maintenance of ovarian cyclic activity in buffalo. Agric. Ric., 153, 5-16.
9. Bucholtz, D. C., Vidwans N. M., Herbosa G. G., Schillo K. K., Foster D. L., 1996. Metabolic interfaces between growth and reproduction. pulsatile luteinizing hormone secretion is dependent on glucose availability. Endocrinol. 137, 601-607.
10. Cooke, R. F., Bohnert D. W., Francisco C. L., Marques R. S., Mueller C. J., Keisler D. H., 2013. Effect of bovine somatotropin administration on growth, physiological and reproductive responses of replacement beef heifers. J. Anim. Sci. 91, 2894-2901.
11. Dupont, J., Scaramuzzi R. J., Reverchon M., 2014. The effect of nutrition and metabolic status on the development of follicles, oocytes and embryos in ruminant. Animal, 1-14.
12. Ettema, J. F., Santos J. E., 2004. Impact of age at calving on location, reproduction, health and income in first-parity Holstein on commercial farms. J. Dairy Sci., 87, 2730-2742.
13. Glibertson, J., Kirkwood R. N., Thacker P. A., 1991. Timing of growth hormone injections and reproduction in Gilts. Candian J. Anim. Sci., 71, 717-723.
14. Gojjam, Y., Tolera A., Mesfin R., 2011. Management optionsto accelerate growth rate and reduce age at first calving in Friesian-Boran crossbred heifers. Trop. Anim. Health Prod., 43, 393-39.
15. Gong, J. G., Baxter G., Bramley T. A., Webb R., 1997. Enhancement of ovarian follicle development in heifers treatment with recombinant bovine somatotropin: a dose-response study. J. Reprod. and Fertil. 110, 91-97.
16. Gong, J. G., Bramley T. A., Webb R., 1991. The effect of recombinant bovine somatotrophin on ovarian follicular function in heifers. Follicular population and peripheral hormones. Biol. Reprod. 45: 941-949.
17. Granger, A. L., Wyatt W. E., Craig W. M., Thompson D. L., Hembiy F. G., 1989. Effects of breed and wintering diet on growth puberty and plasma concentrations of growth hormone and insulin-like growth factor-1 in heifers. Dom. Anim. Endocrinol. 6, 253-263.
18. Haldar, A., Prakash B. S., 2006. Growth hormone-releasing factor (GRF) induced growth hormone advances puberty in female buffaloes. Anim. Reprod. Sci., 92, 254-267.
19. Hall, J. B., Schillo K. K., Fitzgerald B. P., Bradley N. W., 1994. Effect of recombinant bovine somatotropin and diet energy intake on growth, secretion of luteinizing hormone, follicular development and onset of puberty in beef heifers. J. Anim. Sci., 72, 709-718.
20. Khattab, H. M., El-Sayed H. M., Abo El-Nor S. A., El-Alamy H. A., Abd El-Gawad R. M., 2008. Impact of somatotrophin and Monessen on the productive performance of Egyptian dairy buffaloes. Inter. J. Dairy Sci., 3, 1, 11-19.
21. Kirby, C. J., Smith M. F., Keisler D. H., Lucy M. C., 1997a. Follicular function in lactating dairy cows treated with sustained release bovine somatotropin. J. Dairy Sci. 80:273-285.
22. Kirby, C. J., Wilson S. J., Lucy M. C., 1997b. Response of dairy cows treated with bovine somatotropin to a luteolytic dose of prostaglandin F2α. J. Dairy Sci. 80:286–294.
23. Kumar, H., Singh G. D., Pant H. C., 1992. Metabolic profile in relation to hormonal therapy for anoestrous in buffalo heifers. Ind. J. Dairy Sci. 45, 5, 278-280.
24. Nanda, A. S., Brar P. S., Prabhakar S., 2003. Enhancing reproductive performance in dairy buffalo: major constrains and achievements. Reproduction Suppl. 61, 27-36.
25. Naqvi, A. N., Shami S. A., 1999. Comparative performance of early and late maturing Nilli-Ravi buffalo heifers. Asian-Aust. J. of Anim. Sci., 12, 336-340.
26. Patterson, D. J., Perry R. C., Kiracofe G. H., BellowsR. A., Staigmiller R. B., Corah L. R., 1992. Management considerations in heifers development and puberty. J. Anim. Sci., 70, 4018-4035.
27. Pawshe, C. H., Raw K. B., Totey S. M., 1998. Effect of insulin-like growth factor-1 and its interaction with gonadotropin on in vitro maturation and embryonic development, cell proliferation and biosynthetic activity of cumulus-oocyte complexes and granulosa cells in buffalo. Molecular Reprod. and Dev., 29, 277-285.
28. Perera, B. M. A., 1999. Reproduction in water buffalo: Comparative aspects and implications for management. J. Reprod. And Fertil. Suppl. 45, 157-168.

29. Perera, B.M. A., 2008. Reproduction in domestic buffalo. Reprod. Dom. Anim., 43 (Suppl. 2), 200-206.
30. Qureshi, M. S., Habib G., Abdus Samad H., Siddiqui M. M., Ahmad N., Mirajuddin Syed, 2002. Reproduction nutrition relationship in dairy buffaloes: Effect of intake of protein, energy and blood metabolites levels. Asian-Aust. J. Anim. Sci., 15, 330-339.
31. Roche, J. F., Mackey D., Diskin M. D., 2000. Reproductive management of postpartum cows. Anim. Reprod. Sci., 60-61: 703-712.
32. Samadi, E., Blache D., Martin G. B., D'Occhio M. J., 2014. Nutrition, metabolic profile and puberty in Brahman (Bos Indicus) beef heifers. Anim. Reprod. Sci. 146, 3-4,134-142.
33. Sarwar M., Khan M. A., Nisa M., Bhatti S. A., Shahzad M. A., 2009. Nutritional management of buffalo production. Asian-Aust. J. Anim. Sci. 22(7), 1060-1068.
34. SAS, 2000. SAS/GLM User's Guide.Version 8. SAS Inst., Inc., Cary, NC.
35. Singh, C., Madan M. L., 2000. Effect of GnRH on the plasma FSH, LH and estradiol levels at estrous induced with injection of PGF2α and eCG in prepubertial buffaloes. Asian-Aust. J. of Anim. Sci., 11, 78-83.
36. Singh, C., Madan M. L., 1998. Pituitary and gonadal responses to GnRH in prepubertial buffaloes. Asian Aust. J. Anim. Sci., 13, 897-900.
37. Sirotkin, A. V., 2005. Control of reproductive processes by growth hormone: Extra- and inter-cellular mechanism. The Vet. J., 170, 307-317.
38. Terzano, G. M., Barile V. L., Borghese, A., 2012. Overviews on reproductive endocrine aspects in buffalo. J. of Buffalo Sci., 126, 138.
39. Trinder, P., 1969. Determination of blood glucose using 4-aminophenozone as oxygen acceptor. J. Clin. Path., 22:246.
40. Velazquez, M. A., Spicerm L. J., Wathes, D. C., 2008. The role of insulin-like growth factor-1 (IGF-1) in female reproduction, Domest. Anim. Endocrinol. 35, 325-342.
41. Yelich, J. V., Wettemann R. P., Dolezal H. G., Lusby K. S., Bishop D. K., Spicer L. J., 1995. Effect of growth rate on carcass composition and lipid partitioning at puberty and growth hormone, insulin-like factor-1 and metabolities before puberty in beef heifers. J. Anim. Sci., 73, 2390-2405.

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