

*Original Research***Role of Nutrition in Body Weight Gain and Early Onset of Puberty and Sexual Maturity in (HF X Kankrej) Crossbred Heifers**A. J. Dhami<sup>1\*</sup>, K. K. Hadiya<sup>2</sup>, D. V. Chaudhari<sup>3</sup>, P. M. Lunagariya<sup>4</sup>,N. P. Sarvaiya<sup>5</sup> and S. V. Shah<sup>6</sup>

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**Abstract**

Feeding of high plane of nutrition to growing heifers from early age is expected to economically shorten the age at puberty/sexual maturity and thereby the age at first calving. This experiment was initiated on 24 Holstein x Kankrej crossbred heifers of nearly identical age (7-9 months) and body weight (140-156 kg) at University farm. Twelve heifers were managed under routine farm feeding (control) and the rest 12 under ideal optimum feeding regime (treatment) that included extra 1 kg concentrate, 30 g min mix and ad lib dry fodder. Body weight at monthly interval and actual age at puberty/first estrus and sexual maturity/conception were recorded for each animal in both the groups. Ovarian ultrasonography together with blood sampling was carried out at monthly interval from 10 to 18 months of age to study the ovarian dynamics and endocrine changes including thyroid hormones profile. There was gradual, consistent and significant ( $p < 0.05$ ) increase in the monthly body weight gain in heifers of treatment group over control group with advancing age till 18 months of age by which nearly 60% heifers had evinced puberty and were bred. The plasma progesterone and estradiol concentrations monitored from 10 months revealed lower or basal levels till 12-13 months of age with follicles  $< 5$  mm size, and thereafter most of the heifers showed presence of 2-5 follicles  $> 8$  mm in diameter, but ovulatory follicle / CLs were noticed in only a few animals around 15 months, and by about 17-18 months 50 and 75% heifers of control and treatment groups had ovulatory follicles  $> 12$  mm or CL  $> 10$  mm upon USG examination concurrent with elevated plasma progesterone concentrations. Thyroid hormones also showed rising trend with advancing age and showed almost stable profile from 17 months of age onward. One heifer in each group showed consistent true

anestrus with small smooth inactivity ovaries, and one evinced repeat breeding with large flabby flat ovaries, hence were excluded from calculation of pubertal age. The mean age at puberty and sexual maturity (fertile estrus) worked out for remaining 10 animals in each group were  $22.23 \pm 0.45$  and  $24.72 \pm 0.89$  months in control and  $20.40 \pm 0.45$  and  $23.17 \pm 0.60$  months in treatment group, respectively. These data indicated that high plane of nutrition from early age shortened the age at puberty and sexual maturity by nearly two months in HFxK crossbred heifers.

**Key words:** Age at puberty/maturity, Body weight gain, Crossbred heifer, Ovarian ultrasonography, Plane of nutrition, Plasma endocrine profile

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## Introduction

The onset of puberty in dairy animals is one of the economically important reproductive traits. Pubertal development involves physical and behavioural changes that are linked to the activation of the hypothalamic adenohipophyseal gonadal axis (Sisk and Foster 2004). The progression of events is controlled largely by genetic and environmental factors, among which nutrition has a major influence. Signals mediating nutritional and metabolic information are perceived largely at the level of the hypothalamus and are likely integrated in structural and cellular networks that control various neuroendocrine functions, including puberty (Schneider 2004). The birth weight of female Holstein Friesian x Kankej (HFxK, inter-se) calves is recorded around 24 kg with daily body weight gain of around 450 g under ideal feeding and managemental conditions, so that they attain the age at puberty at around 16-18 months and age at maturity around 20-22 months with age at first calving (AFC) of 30-32 months (Anon 2018). However, under field conditions, crossbred heifers attain puberty very late around 2.5 to 3.0 years of age mainly because of not attaining proper body weight (240-275 kg) and thereby give first calf at the age of 3.5 to 4.0 years. The main constraint is poor scientific practices and negligence of farmers, non-availability of quality dry and green fodder and concentrate mixture, mineral mixture, salt etc. leading to malnutrition or under nutrition in the field. The nutritional intervention is expected to enhance puberty and AFC in these animals (Anon 2018). Hence, this experiment was initiated with the hypothesis that the special nutritional care of heifers if given right from the age of 6-9 months, they would gain body weight and confirmation optimum to exhibit estrus and conceive by 20-22 months of age. Thus, the objective of this study was to evaluate the role of optimum feeding on growth rate, ovarian dynamics and hormonal profile in prepubertal HF x K heifers towards reducing the age at puberty/sexual maturity.

## Materials and Methods

The crossbred (Holstein Friesian x Kankrej) heifers (N=24) around 7-9 months of age and nearly identical body weight (140-156 kg) were selected from the crossbred herd of Livestock Research Station, AAU, Anand in two phases during the year 2016 and 2017. Experiment was approved by the Institutional Animal Ethics Committee for use of farm animals. The heifers were randomly divided into two equal groups, one half of them (n=12) were kept under routine farm feeding schedule) control group (and rests 12 were managed under ideal optimum feeding practices) treatment group) that included extra 1 kg concentrate (22% CP), 30 g min mix and ad lib dry fodder. Animals of both the groups were studied for their physiological parameters such as growth rate, age at puberty, sexual maturity and conception rate. Evaluation of ovarian development and dynamics was done using monthly per rectal palpation and ultrasound monitoring of ovaries and genitalia from 10 month still 18 months of age, along with blood sampling for endocrine studies. For ultrasonography of ovaries, the trans-rectal linear array transducer was used with 5.0-7.5 MHz frequency (M-5 Vet, Mindray) as per Ginther et al (1995). Typical images (sonographs) were frozen on screen and printed using videographic thermal printer (Brothers, India). The work was continued over 18 months for evaluation of nutritional interventions on puberty/maturity and subsequent related reproductive parameters.

Blood plasma samples were stored in deep freeze at -20°C with a drop of merthiolate (0.1%) until analyzed. The levels of plasma progesterone, estradiol, and thyroid hormones T<sub>3</sub>, T<sub>4</sub> were estimated using standard RIA techniques employing CT RIA kits of Beckman and Coulter supplied by Immunotech-SAS, France. The sensitivity of the progesterone and estradiol assays was 0.1 ng/ml and 4.0 pg/ml, respectively. The intra- and inter-assay coefficients of variation for progesterone assay were 5.4 and 9.1 per cent, while the same for estradiol assay were 15.1 and 14.4 per cent, respectively. The sensitivity of T<sub>3</sub> and T<sub>4</sub> assay was 0.24 and 0.50 ng/ml, respectively.

The means and standard errors of reproductive parameters were calculated using descriptive statistics and were compared between groups by paired 't' test. The data on endocrine profile was statistically analyzed using ANOVA, DMRT and 't' test employing SPSS software version 20.0 (Snedecor and Cochran 1994).

## Results and Discussion

### *Body Weight and Growth Rate in Control and Treatment Groups*

The experiment was initiated on HF x K crossbred heifers at an average age of 8.5 months with body weight of 140 to 156 kg. There was gradual consistent and significant (p<0.05) gain in the body weight measured

at monthly interval in heifers of treatment group over control group with advancing age till 17.5 months of age (Table 1), by which nearly 60% heifers had evinced puberty and were bred.

**Table 1:** Effect of high plane of nutrition on body weight gain in growing HFxK crossbred heifers

Age in Months	Mean monthly body weight (kg)		Difference in weight
	Control (n=12)	Treatment (n=12)	
08.5	148.83±10.14 <sup>a</sup>	148.10±11.46 <sup>a</sup>	-0.72
09.5	154.47±9.63 <sup>a</sup>	156.05±11.45 <sup>a</sup>	+1.58
10.5	174.15±9.85 <sup>ab</sup>	185.96±11.98 <sup>ab</sup>	+11.81
11.5	185.63±10.03 <sup>b</sup>	202.13±12.62 <sup>bc</sup>	+16.50
12.5	200.37±9.39 <sup>bc</sup>	222.58±12.06 <sup>bcd</sup>	+22.22
13.5	214.27±9.19 <sup>cd</sup>	*237.52±12.27 <sup>cd</sup>	+23.25
14.5	227.13±8.54 <sup>de</sup>	*256.55±12.23 <sup>de</sup>	+29.18
15.5	249.13±8.52 <sup>ef</sup>	*281.63±12.94 <sup>ef</sup>	+32.50
16.5	263.13±8.23 <sup>fg</sup>	*294.37±12.81 <sup>f</sup>	+30.67
17.5	277.95±8.67 <sup>g</sup>	*310.23±13.65 <sup>f</sup>	+32.28

\*P<0.05 between groups, Means with different superscripts within column differ significantly.

Environmental factors, nutritional status, body condition score, and pre- and post- weaning weight gain are important factors to reduce age at puberty (Patterson *et al* 1992). Post-weaning nutrition plays a key role in the determination of age at puberty (Thallman *et al* 1999). In this respect, the main reasons for late onset of puberty are the seasonal forage production, poor pasture management, and lack of dietary supplementation during growth. It is possible to reduce the age at puberty as demonstrated in studies in which first breeding occurred at 18 months of age (Eler *et al* 2002). Similar results have been reported by De Lucia *et al* (2002) and Monteiro *et al* (2013). Recently, Pinheiro *et al* (2010) studied a line of Nellore heifers selected for weight similar to the NeS line. The animals were kept in a feedlot and were given nutritional supplementation for 140 days after weaning. In a literature review, 50% of half-blood *Bos indicus* heifers were able to breed at 15 months of age when fed a diet designed to obtain high daily weight gain (Lanna 2004). However, a high proportion of prepubertal heifers were observed in *Bos indicus* herds even among animals older than 2 years, probably because of a low body weight and lack of fat reserves (Lanna 2004). These reports support the present findings on HF X K heifers.

### Ovarian Dynamics and Endocrine Profile

The plasma progesterone and estradiol concentrations monitored at monthly intervals from average 10 months of age revealed lower or basal levels till 12-13 months of age and thereafter most of the heifers showed presence of 2-5 follicles >8 mm in diameter, but ovulatory follicle / CLs were noticed in only a few animals around 15 months, and by about 17-18 months 50 and 75% heifers of control and treatment groups had ovulatory follicles >12 mm or CL >10 mm upon USG examination concurrent with elevated plasma progesterone concentrations (Table 2, Fig. 1). The observations have also determined that ovarian follicles

in prepubertal heifers grow and regress in waves, following the same process as in adult animals. However, in adult animals, these follicles develop to the ovulatory stage (Hopper *et al* 1993, Bergfeld *et al* 1994). Ovaries of prepubertal heifers contain at least one follicle greater than 7 mm in surface diameter by 100 days prior to the first ovulation (Evans *et al.*, 1994). In a previous study, low dietary intake reduced the diameter and persistence of follicles during the estrous cycle of post-pubertal beef heifers and tended to increase the proportion of estrous cycles with three dominant follicles (Murphy *et al* 1991).

There is an increase in secretion of LH as puberty approaches, particularly during the last 50 days prior to the pubertal ovulation (Day *et al* 1986). These increases in LH can be delayed when heifers are subjected to restrictions in dietary energy intake (Kurz *et al* 1990). On the basis of these results and of our current knowledge of the role of LH in regulating growth of ovarian follicles, we predicted that waves of follicular growth during pubertal development would differ, in relation to the patterns of LH to which the ovaries are exposed, when heifers were fed high as compared to low amounts of energy during sexual maturation. Our results indicate that dominant ovarian follicles have a larger diameter as heifer approaches puberty and lower levels of dietary energy alters the chronological pattern of ovarian follicular development.

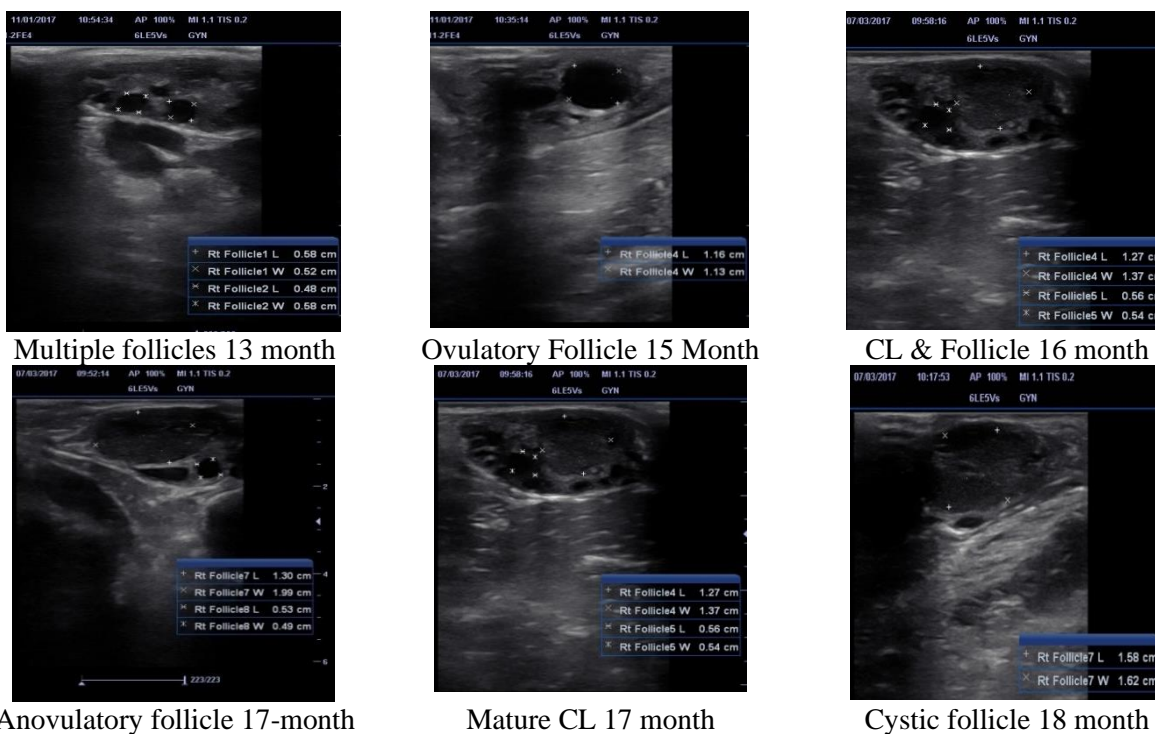
The primary difference observed in the current study was that heifers fed the diet with low energy content had a delay in chronology of development of dominant follicles in comparison to heifers fed the diet containing greater energy. An alternate view of this would be that heifers fed the diet with the greater energy had accelerated development of maturational events associated with puberty. In previous studies, maximum diameter of dominant follicles was smaller in post-pubertal heifers maintained on a restricted-energy diet when compared to that in heifers maintained on a diet of moderate energy content (Murphy *et al* 1991; Rhodes *et al* 2003).

As the time of puberty approached, dominant ovarian follicles of greater diameter developed in heifers. This increase allowed for the greater secretion of estradiol by these follicles, which would have resulted from the decreased negative feedback of estradiol on secretion of LH prior to puberty (Day *et al* 1986, Wolfe *et al* 1991). The hypothalamic-pituitary axis is hypersensitive to the negative feedback of estradiol, which in turn limits secretion of LH prior to the onset of puberty. The prepubertal decline in negative feedback of estradiol on secretion of LH is followed by a period of positive feedback post-puberty. As sensitivity to estradiol lessens, there is a subsequent increase in frequency of LH pulses, particularly during the last 30 days preceding puberty (Bergfeld *et al* 1994). Our findings indicate that continuous increase of ovarian size occurred until heifers reached puberty. When heifers were classified according to pubertal status, pubertal heifers had a larger follicle diameter than non-pubertal heifers which were in accordance with previous reports of Andersen *et al* (1991), Ferreira *et al* (1999), Holm *et al* (2009) and Monterio *et al* (2013).

**Table 2:** Influence of age and nutritional supplementation on plasma profile of sex steroids in growing HF x K crossbred heifers

Age (month)	Progesterone (ng/ml)			Estradiol (pg/ml)		
	Control (n=12)	Treatment (n=12)	Pooled (n=24)	Control (n=12)	Treatment (n=12)	Pooled (n=24)
10	0.18 <sup>ab</sup> ±0.08	0.17 <sup>a</sup> ±0.09	0.17 <sup>a</sup> ±0.06	9.27 <sup>a</sup> ±2.26	8.38 <sup>a</sup> ±2.79	8.82 <sup>a</sup> ±1.76
11	0.13 <sup>a</sup> ±0.02	0.12 <sup>a</sup> ±0.02	0.12 <sup>a</sup> ±0.01	11.38 <sup>a</sup> ±3.58	7.98 <sup>a</sup> ±1.55	9.68 <sup>a</sup> ±1.94
12	0.12 <sup>a</sup> ±0.02	0.15 <sup>a</sup> ±0.03	0.14 <sup>a</sup> ±0.01	14.63 <sup>a</sup> ±3.76	7.32 <sup>a</sup> ±1.18	10.98 <sup>a</sup> ±2.07
13	0.15 <sup>ab</sup> ±0.02	0.15 <sup>a</sup> ±0.02	0.15 <sup>a</sup> ±0.01	23.37 <sup>a</sup> ±5.28	28.12 <sup>b</sup> ±6.35	25.74 <sup>b</sup> ±4.07
14	0.15 <sup>ab</sup> ±0.02	0.33 <sup>a</sup> ±0.13	0.24 <sup>a</sup> ±0.07	55.96 <sup>c</sup> ±8.41	49.75 <sup>c</sup> ±4.39	52.85 <sup>d</sup> ±4.69
15	0.09 <sup>a</sup> ±0.02	0.51 <sup>a</sup> ±0.18*	0.30 <sup>ab</sup> ±0.10	70.75 <sup>d</sup> ±6.65	77.42 <sup>d</sup> ±6.50	74.08 <sup>e</sup> ±4.60
16	0.22 <sup>b</sup> ±0.09	0.28 <sup>a</sup> ±0.11	0.25 <sup>a</sup> ±0.07	40.75 <sup>bc</sup> ±5.86	38.21 <sup>bc</sup> ±4.86	39.48 <sup>c</sup> ±3.73
17	0.47 <sup>b</sup> ±0.15	0.72 <sup>a</sup> ±0.28	0.60 <sup>b</sup> ±0.16	44.67 <sup>bc</sup> ±4.69	49.25 <sup>c</sup> ±8.33	46.96 <sup>cd</sup> ±4.70
18	1.02 <sup>c</sup> ±0.25	1.49 <sup>b</sup> ±0.34**	1.26 <sup>c</sup> ±0.25	39.46 <sup>b</sup> ±4.21	51.76 <sup>c</sup> ±7.72	45.61 <sup>cd</sup> ±4.48

\*P<0.05, \*\*P<0.01 between subgroups; Means bearing uncommon superscripts within the column differ significantly (p<0.05).



**Fig. 1:** Sonographic visualization of ovarian functions in pubertal HF x K crossbred heifers

Circulating concentrations of estradiol are low initially but increases with age as the prepubertal heifer matures, particularly over the last 12 weeks before first ovulation (Evans *et al* 1994, Melvin *et al* 1999). However, the largest increase occurs in a few days prior to first ovulation (Evans *et al* 1994). First ovulation in heifers is often not associated with estrus, and the subsequent corpus luteum is smaller than in a normal cycle and may be short-lived (Evans *et al* 1994). This short cycle is followed by estrus and a normal, full-length luteal phase. Increased estrogen secretion eventually causes a preovulatory LH surge and first

ovulation. Estradiol appears to be the preeminent negative feedback agent on LH secretion, operating indirectly through other neuronal systems.

Thyroid hormones also showed rising trend with advancing age and showed almost stable profile from 17 months of age onward as of adult animals (Table 3). Normal growth and development occur in animals only in the presence of thyroid hormone, indicating that thyroid hormones are necessary for normal growth and play a permissive role in growth regulation. Thyroid hormones directly influence growth by altering biochemical reactions; many of them influence size of specific tissues and organs. These hormones affect body mass and dimensions primarily by altering skeletal and/or nitrogen metabolism. In addition, thyroid hormones play a permissive role in growth regulation and are essential for maintenance of the basal metabolic rate (Ingole *et al* 2012, Habeeb *et al* 2016).

**Table 3:** Influence of age and nutritional supplementation on plasma profile of thyroid hormones in growing HF x K crossbred heifers

Age (month)	Tri-iodothyronine (ng/ml)			Thyroxine (ng/ml)		
	Control (n=12)	Treatment (n=12)	Pooled (n=24)	Control (n=12)	Treatment (n=12)	Pooled (n=24)
10	1.73 <sup>ab</sup> ±0.11	1.84 <sup>ab</sup> ±0.17	1.79 <sup>abc</sup> ±0.10	18.00 <sup>a</sup> ±2.17	18.42 <sup>a</sup> ±2.36	18.21 <sup>a</sup> ±1.57
11	1.88 <sup>ab</sup> ±0.25	1.82 <sup>abc</sup> ±0.14	1.85 <sup>bc</sup> ±0.14	29.00 <sup>bc</sup> ±3.26	29.29 <sup>b</sup> ±3.88	29.15 <sup>bc</sup> ±2.48
12	1.50 <sup>a</sup> ±0.11	1.48 <sup>a</sup> ±0.08	1.49 <sup>a</sup> ±0.07	28.25 <sup>ab</sup> ±3.51	29.08 <sup>b</sup> ±2.88	27.67 <sup>b</sup> ±2.24
13	1.70 <sup>ab</sup> ±0.14	1.91 <sup>bcd</sup> ±0.16	1.80 <sup>bc</sup> ±0.11	33.50 <sup>bc</sup> ±3.51	31.75 <sup>bc</sup> ±3.02	32.83 <sup>bcd</sup> ±2.27
14	1.69 <sup>ab</sup> ±0.17	1.98 <sup>cd</sup> ±0.16	1.83 <sup>bc</sup> ±0.12	35.50 <sup>bc</sup> ±4.40	35.25 <sup>bcd</sup> ±1.63	35.38 <sup>cd</sup> ±2.29
15	1.55 <sup>a</sup> ±0.15	1.54 <sup>ab</sup> ±0.08	1.55 <sup>ab</sup> ±0.08	35.08 <sup>bc</sup> ±3.30	40.33 <sup>cde</sup> ±2.72	37.70 <sup>d</sup> ±2.16
16	1.61 <sup>a</sup> ±0.11	1.70 <sup>abc</sup> ±0.07	1.65 <sup>ab</sup> ±0.07	38.00 <sup>c</sup> ±4.19	41.32 <sup>de</sup> ±3.49	39.67 <sup>d</sup> ±2.69
17	1.92 <sup>ab</sup> ±0.14	2.05 <sup>cd</sup> ±0.17	1.98 <sup>cd</sup> ±0.11	48.83 <sup>d</sup> ±3.69	46.25 <sup>ef</sup> ±3.15	47.54 <sup>e</sup> ±2.39
18	2.14 <sup>b</sup> ±0.16	2.27 <sup>d</sup> ±0.13	2.21 <sup>d</sup> ±0.10	52.67 <sup>d</sup> ±4.50	51.25 <sup>f</sup> ±3.67	51.96 <sup>e</sup> ±2.84

Means bearing uncommon superscripts within the column differ significantly ( $p < 0.05$ ).

### Actual Age at Puberty and Sexual Maturity in Crossbred Heifers

Among 12 heifers each covered in control and treatment groups, one in each group showed consistent anestrus with small smooth inactivity ovaries till 24 months of age. Similarly one heifer in each group evinced repeat breeding with large flabby flat ovaries though exhibited puberty in time and hence were excluded from final calculation of pubertal age. The mean age at puberty and sexual maturity (fertile estrus) worked out for remaining 10 animals in each group were 22.23±0.45 and 24.72±0.89 months in control and 20.40±0.45 and 23.17±0.60 months in treatment group, respectively (Table 4).

These data clearly indicate that high plane of nutrition from early age shortens the age at puberty and sexual maturity by nearly two months in HFxK crossbred heifers. Heifers reached puberty at heavier weight and at an earlier age. According to Roberts *et al* (2009) and Monteiro *et al* (2013), age at puberty is more affected by the growth rate and body weight gain. These results agreed with other studies reporting an age

at puberty of 22-24 months and age at first calving of 32-34 months (Manzano *et al* 1993; Souza *et al* 1995). The age of heifers at puberty is directly related to weight and body condition. According to Romano *et al* (2007), heifers that consumed more energy and had a higher daily weight gain rate reached puberty at younger age. Similarly, in the present study, pubertal heifers were heavier and younger than non-pubertal animals. In *Bos taurus* beef heifers, the onset of puberty is defined as the age when the animals reaches approximately 60% of its mature weight (Romano *et al* 2007). This percentage is approximately 65% for *Bos indicus* heifers (Monteiro *et al* 2013).

**Table 4:** Mean age at puberty and sexual maturity in control and nutritional supplemented HFxK crossbred heifers

Reproductive Status	Control group		Treatment group	
	Days	Months	Days	Months
Puberty	680.73±11.97	22.23±0.45	621.30±15.30	20.40±0.45
Maturity	752.00±26.90	24.72±0.89	703.11±18.43	23.17±0.60

## Conclusion

High plane of nutrition to growing crossbred (HFxK) heifers right from early age was beneficial in terms of significant gain in body weight (25-35 kg) and reducing the age of onset of puberty and sexual maturity (by 2-3 months) compared to routine farm fed groups. The plasma progesterone and estradiol concentrations monitored at monthly intervals from 10 months of age revealed presence of 2-5 follicles >8 mm in diameter around 12-13 months of age, and by about 17-18 months 50 and 75% heifers of control and treatment groups had ovulatory follicles >12 mm or CL >10 mm upon USG examination concurrent with elevated plasma progesterone. Thyroid hormones also showed rising trend with advancing age and reached plateau around 17 months of age. Thus, optimally fed crossbred heifers right from early age attained puberty and sexual maturity 2-3 months earlier than routine farm fed heifers.

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## Conflict of Interest

All authors declare that they do not have conflict of interest.

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