RESEARCH ARTICLE

Ovarian Follicular Dynamics, and Hormonal and Biochemical Profile in Post-Pubertal and Postpartum Jaffrabadi Buffaloes

Rupesh J Raval[1]*, Kiran H Parmar[1], Arjun J Dhami[2], Fulabhai S Kavani[2]

Abstract

Jaffrabadi buffalo is one of the heaviest buffalo breeds of the world (800 kg) and is known for poor reproductive efficiency. There is a scarcity of normal physiological data regarding reproductive parameters of Jaffrabadi buffalo. The primary objective of this study was to elucidate ovarian follicular dynamics, hormonal and biochemical profiles in 10 post-pubertal heifers (n=5 each of age 42±2 and 48±2 months) and five >90 days postpartum lactating buffaloes. Ultrasound monitoring of ovaries of animals was done at weekly interval and all follicles with diameter <4 mm (small follicles), 4-8 mm (medium follicles) and >8 mm (large follicles) as well as CLs identified were recorded. Blood samples were also collected soon after each USG from all animals for assay of hormonal and biochemical profiles. The findings revealed that the average number of small, medium and large follicles, total number of follicles per ovary as well as diameters of largest and subordinate follicles varied greatly among periods between three groups. The size of corpus luteum ranged from 13.7 to 21.2 mm, 13.7 to 23.9 mm and 15.3 to 22.7 mm, respectively, in Group-I, II and III. In the first week of study, significantly higher ($p < 0.05$) average numbers of small follicles in Group-I and III, and medium follicles and total numbers of follicles in Group-III were observed as compared to other groups. The diameter of largest follicles and subordinate follicles were non-significantly different among all groups at all periods of the study. Plasma estrogen levels were significantly higher ($p < 0.05$) in Group-I as compared to Group-III and Group-II on first and second week, however, it increased significantly ($p < 0.05$) in Group-II and I on fourth week of study period. Plasma insulin concentrations were significantly ($p < 0.01$) lower in buffaloes (Group-III) than in heifers (Group-I and II) throughout the study period, while plasma total cholesterol and total protein levels were higher in Group-III on 3rd to 5th weeks of study. Further, there were no significant differences in the plasma FSH, LH, progesterone and blood glucose levels in animals of three groups between periods at any of the week. The ultrasonographic and endocrine profile suggested establishment of cyclicity with silent ovulation. However, the behavioural estrus was still not manifested by these post-pubertal heifers and postpartum lactating buffaloes. These results are helpful to diagnose anovulatory conditions and/or true anestrus and initiate estrus synchronization protocols for early conception in Jaffrabadi buffaloes.

Keywords: Biochemical profile, Hormonal profile, Jaffrabadi buffalo, Ovarian follicular dynamics, Post-pubertal heifers.

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Introduction

Jaffrabadi buffalo is one of the heaviest buffalo breeds of the world (800 kg) and is a native of Saurashtra region of Gujarat, India. The breed is known for poor reproductive efficiency. Ovarian dynamics and peripheral circulatory concentrations of various hormones and metabolites are associated directly or indirectly with reproduction. The different phases of reproductive cycle are regulated by intricate sequential events and interactions between hypothalamic releasing hormones, and hormones of pituitary and gonads. Lack of integration or imbalance at any phase of the sequence may result in reproductive failure. Hence, such information help to solve problem of silent estrus, poor expression of behavioural estrus, late initiation of cyclicity postpartum etc. (Mondal *et al.,* 2007). Reproductive ultrasound has emerged as an accurate, non-invasive and reliable technique to elucidate follicular patterns of development, evaluation of ovarian function, CL development and has been successfully used in animal reproduction (Presicce *et al.*, 2005). Knowledge of follicular patterns and folliculogenesis are necessary to successfully implement both estrus synchronization and superovulation protocols, as well to diagnose anovulatory conditions and anestrus in female buffaloes and thereby to ameliorate the same. There is a scarcity of normal physiological data regarding reproductive parameters of Jaffrabadi buffalo. The primary objectives of this study were therefore to elucidate

[1]College of Veterinary Science & AH, Junagadh Agricultural University, Junagadh - 362 001, India

[2]College of Veterinary Science & AH, Anand Agricultural University, Anand - 388 001, India

Corresponding Author: Rupesh J. Raval, College of Veterinary Science & AH, Junagadh Agricultural University, Junagadh - 362 001, India, e-mail: rupeshraval@rediffmail.com

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ovarian follicular dynamics, and hormonal and biochemical profiles in post-pubertal heifers and >90 days postpartum lactating Jaffrabadi buffaloes.

Materials and Methods

The study was carried out under tropical climate following approval of the Institutional Animal Ethics Committee on 10 post-pubertal Jaffrabadi heifers (Group I, age 42±2 months; Group II, age 48±2 months) and five >90 days postpartum lactating buffaloes (Group III) maintained under standard management practices at the University Farm, JAU, Junagadh, India. To monitor the ovarian dynamics, the ultrasonography was performed using real-time B-mode ultrasound scanner equipped with a 5.0-7.5 MHz rectal probe (DB355M, IMAGO.S, ECM, France) at weekly interval for first 5 weeks and thereafter fortnightly on 7th and 9th week. All follicles with diameter <4 mm (small follicles), 4-8 mm (medium follicles) and >8 mm (large follicles) as well as CLs (Malik, 2005) identified were recorded.

Blood sampling in K_3EDTA vaccutainers was performed along with ultrasound scanning of all animals at above intervals, and plasma was stored at –20°C until analyzed. Plasma concentrations of follicle stimulating hormone (FSH) (Cloud-Clone Corp., USA), luteinizing hormone (LH), estrogen and insulin (MyBioSource, Inc., USA) were determined using Enzyme Linked Immuno Sorbent Assay (ELISA) kits as per manufacturers' instructions. Plasma progesterone was determined by employing standard Radio Immuno Assay (RIA) technique and kits of Immunotech-SAS, France. The blood glucose levels were determined immediately in freshly collected whole blood samples using Glucometer. Plasma total cholesterol and total protein levels were determined by CHOD/PAP and Biuret method, respectively, using kits procured from Diatek Healthcare Pvt. Ltd., Kolkata, India.

Data on ovarian dynamics were analyzed with normal distribution and homogeneous variance by parametric one-way analysis of variance (ANOVA) followed by Tukey's HSD test. All graphs were prepared with Graphpad prism 9.0.1 and blood profile was analyzed statically by General Linear Model using SPSS and the mean differences between periods within the group were compared by Duncan's multiple range test, and the period wise group differences by paired 't' test (Snedecor and Cochran, 1994).

Results and Discussion

In the present study, irrespective of groups and periods, we found average small follicles population per ovary ranging from 2.80±0.25 to 4.40±0.24, medium follicles population

Table 1: Average (± SE) number of follicle population and diameters of largest and subordinate follicles during different periods of monitoring in post-pubertal heifers (Gr I & II) and lactating Jaffrabadi buffaloes (Gr III)

Group	*Days of sampling*	*No. of Small Follicles*	*No. of Medium Follicles*	*No. of Large Follicles*	*Total No of follicle*	*Diameter of largest follicle*	*Diameter of subordinate Follicle*
Gr-I (Post-pubertal Jaffrabadi heifers 42 ± 2 Months; n=5)	1st week	3.80 ± 0.12^{a}	1.90 ± 0.19^{b}	1.50 ± 0.32	7.20 ± 0.51^{a}	9.98 ± 0.59	7.94 ± 0.75
	2nd week	3.20 ± 0.30	1.90 ± 0.19	1.40 ± 0.16	6.50 ± 0.63	10.03 ± 0.29	8.39 ± 0.36
	3rd week	3.50 ± 0.22	2.40 ± 0.19	2.10 ± 0.10	8.00 ± 0.35	11.69 ± 0.67	10.02 ± 0.38
	4th week	4.10 ± 0.24	2.60 ± 0.19	1.80 ± 0.12	8.50 ± 0.45	11.29 ± 0.99	8.99 ± 0.36
	5th week	4.40 ± 0.24	2.70 ± 0.12	2.20 ± 0.20	9.30 ± 0.20^{a}	11.76 ± 0.85	10.08 ± 0.39
	7th week	3.90 ± 0.37	2.70 ± 0.34	1.90 ± 0.19	8.50 ± 0.57	12.19 ± 0.70	9.67 ± 0.65
	9th week	3.20 ± 0.20	2.20 ± 0.25	1.90 ± 0.10	7.30 ± 0.49	10.85 ± 0.27	9.11 ± 0.41
Gr-II (Post-pubertal Jaffrabadi heifers 48 ± 2 months; n=5)	1st week	3.00 ± 0.16^{b}	1.90 ± 0.10^{b}	0.90 ± 0.33	5.80 ± 0.25^{b}	9.21 ± 1.19	7.10 ± 0.71
	2nd week	2.90 ± 0.40	2.50 ± 0.16	1.00 ± 0.35	6.40 ± 0.60	9.38 ± 1.03	7.96 ± 0.58
	3rd week	3.00 ± 0.32	2.20 ± 0.20	1.50 ± 0.22	6.70 ± 0.46	11.22 ± 1.05	8.39 ± 0.68
	4th week	3.60 ± 0.19	2.80 ± 0.25	1.60 ± 0.10	8.00 ± 0.42	10.60 ± 0.37	8.77 ± 0.30
	5th week	3.30 ± 0.41	2.50 ± 0.27	1.70 ± 0.20	7.50 ± 0.72^{b}	11.56 ± 0.57	9.10 ± 0.81
	7th week	3.20 ± 0.30	2.20 ± 0.12	1.60 ± 0.24	7.00 ± 0.61	10.20 ± 0.58	8.17 ± 0.46
	9th week	2.80 ± 0.25	2.20 ± 0.20	1.50 ± 0.16	6.50 ± 0.32	10.63 ± 0.42	8.38 ± 0.25
Gr-III (Post-partum lactating Jaffrabadi buffaloes; n=5)	1st week	3.90 ± 0.33^{a}	2.70 ± 0.20^{a}	1.50 ± 0.32	8.10 ± 0.62^{a}	11.04 ± 1.05	9.14 ± 1.13
	2nd week	3.60 ± 0.43	2.40 ± 0.19	1.10 ± 0.19	7.10 ± 0.53	9.86 ± 0.55	7.71 ± 0.34
	3rd week	3.70 ± 0.20	2.40 ± 0.29	1.60 ± 0.19	7.70 ± 0.25	11.81 ± 0.34	8.43 ± 0.52
	4th week	3.90 ± 0.43	2.40 ± 0.29	1.10 ± 0.43	7.40 ± 0.73	10.05 ± 0.88	7.98 ± 0.76
	5th week	3.40 ± 0.19	2.10 ± 0.19	1.70 ± 0.20	7.20 ± 0.44^{b}	11.77 ± 0.82	8.86 ± 0.59
	7th week	3.90 ± 0.19	2.30 ± 0.12	1.60 ± 0.29	7.80 ± 0.46	11.51 ± 0.68	8.95 ± 0.95
	9th week	3.30 ± 0.20	2.20 ± 0.12	1.50 ± 0.22	7.00 ± 0.45	10.56 ± 0.72	8.55 ± 0.56

Means bearing uncommon superscripts (a, b) differ significantly ($p < 0.05$) for a particular week between three groups

from 1.90±0.10 to 2.80±0.25, large follicles population from 0.90±0.33 to 2.20±0.20, total number of follicular population per ovary 5.80±0.25 to 9.30±0.20, and diameter of largest follicle 9.21±1.19 to 12.19±0.70 mm and diameter of subordinate follicle 7.10±0.71 to 10.08±0.39 mm (Table 1). Ultrasound monitoring of ovaries of animals revealed significantly higher ($p < 0.05$) average numbers of small follicles on first week in Group-I and Group-III (3.80±0.12, 3.90±0.33, respectively) as compared to Group-II (3.00±0.16). Similarly, the average medium follicles population was significantly higher ($p < 0.05$) in Group-III (2.70±0.20) as compared to Group-I (1.90±0.19) and Group-II (1.90±0.10) on first week of monitoring. However, the average number of large follicles did not differ significantly between three groups (Table 1, Fig. 1). The average number of total follicles was significantly higher ($p < 0.05$) in Group-III (8.10±0.62) as compared to Group-I (7.20±0.51) and Group-II (5.80±0.25) on first week of monitoring. On the contrary, at fifth week the average number of total follicles increased significantly ($p < 0.05$) in Group-I (9.30±0.20) as compared to Group-II (7.50±0.72) and Group-III (7.20±0.44). Further, the diameter of largest follicles and subordinate follicles were non-significantly different among all groups at all periods of the study (Table 1, Fig. 2).

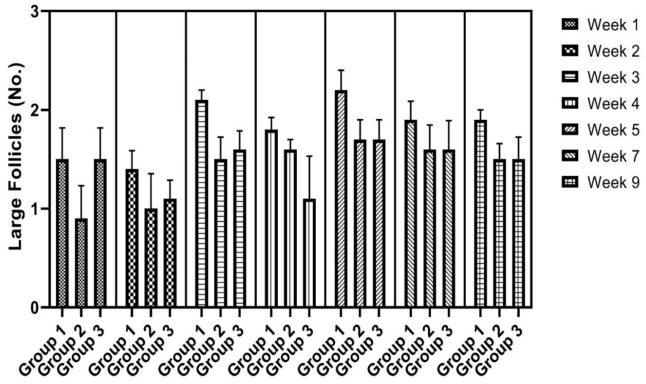

Fig. 1: Average number of large follicles on ovaries of Jaffrabadi animals of three groups at different intervals

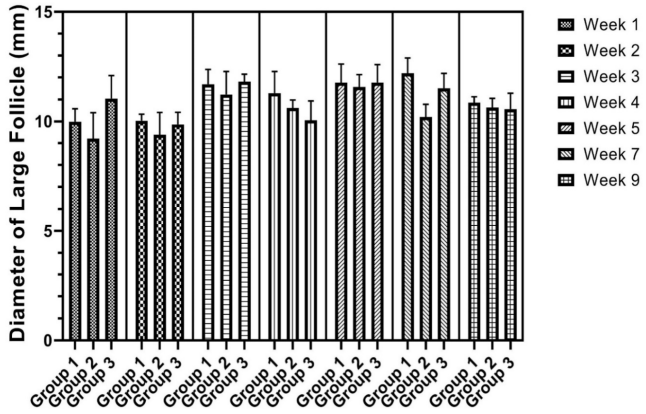

Fig. 2: Average diameter of largest follicles on both ovaries of Jaffrabadi animals of three groups at different intervals

Kumar *et al.* (1997) also reported the average number of follicles visible on the surface of each ovary in buffalo as 5.2±1.0 which was lower than our findings. Similarly Potteupenjera *et al.* (2018) reported lower small follicles (2.13±0.06) and medium follicles (1.63±0.03) in Murrah buffalo treated with buserelin during non-breeding season than in untreated animals of the present study, but the maximum diameter of largest (10.76±0.25 mm) and subordinate (8.33±0.33 mm) follicles in treated Murrahs were close to our observations in Jaffrabadi buffaloes. Raval *et al.* (2020) also reported more or less very similar findings in Jaffrabadi buffaloes treated with double Ovsynch protocol, irrespective of groups and periods, which were close to our normal physiological results of present study without any treatment.

In the present study, numbers of different size of follicles, total follicles and diameters of largest and subordinate follicles gradually yet non-significantly increased till 5th week of monitoring in all three groups. This may be associated with ovarian massage/simulation being induced by per rectal examination and ultrasonography. The non-significantly greater number of ovarian follicles observed in lactating buffaloes as compared to post-pubertal Jaffrabadi heifers may be due to difference in cyclical/acyclic and nutrition status of animals. The numbers and diameters of follicles were not consistent during the study period of two months in all three apparently acyclic groups. Difference between follicular numbers during the cycle is generally attributed to the emergence of small follicles (beginning of wave) and their growth and regression. Follicular development and atresia have been described as a natural phenomenon in ovarian function in Surti buffalo (Danell, 1987). Pubertal heifers had follicular growth similar to adult Mediterranean buffaloes, however, growth rates were slower and the size of the dominant follicle was reported to be smaller in heifers (Presicce *et al.*, 2004, 2005).

In the present study, irrespective of groups and periods, the size of corpus luteum (CL) ranged from 13.7 to 21.2 mm, 13.7 to 23.9 mm and 15.3 to 22.7 mm, respectively, in Group-I, II and III (Fig. 3). We found comparatively large size of CL in postpartum buffaloes as compared to post-pubertal heifers. In majority of animals estrus behaviour was not detected during the study period even though they frequently had large follicles (dominant follicles). Further, the ultrasound monitoring of ovaries at different period of study indicated presence of CL in most of the animals suggesting silent ovulation in them. The etiology of silent estrus /ovulation appears to be complex as 37.2-46.2% of buffaloes with good body condition score showed sub-estrus throughout the year reflecting a genetic predisposition (Devkota *et al.*, 2012). The size and presence of a CL are important to assess cyclicity of the animal to diagnose anovulatory conditions and/or

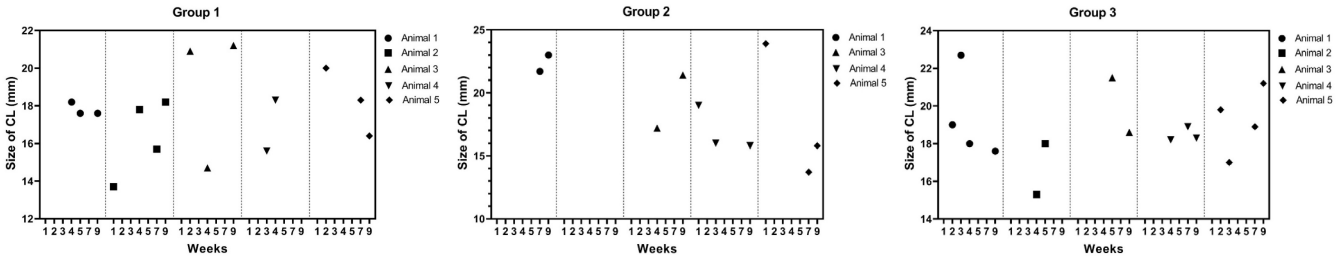

Fig. 3: Size of CL in Post-pubertal Jaffrabadi heifers of Group I (42±2 months) and Group II (48±2 months) and Postpartum lactating buffaloes of Group III at different intervals

Table 2: Mean (± SE) plasma profile of various hormones and biochemical constituents during different periods of monitoring in post-pubertal heifers (Gr I & II) and postpartum lactating Jaffrabadi buffaloes (Gr III)

Group	*Days of sampling*	*FSH (ng/ml)*	*LH (ng/ml)*	*Estrogen (ng/ml)*	*Progesterone (ng/ml)*	*Insulin (pg/ml)*	*Glucose (mg/dl)*	*Cholesterol (mg/dl)*	*Total Protein (g/dl)*
Gr-I (Post-pubertal Jaffrabadi heifers 42 ± 2 months; n=5)	1st week	4.21 ± 0.80	0.59 ± 0.09	10.91 ± 1.93^{a}	0.22 ± 0.12	147.65 ± 5.74^{a}	71.60 ± 1.86	88.20 ± 10.48	6.44 ± 0.25
	2nd week	4.80 ± 0.73	0.80 ± 0.25	9.78 ± 1.46^{a}	0.10 ± 0.00	142.41 ± 2.33^{a}	69.00 ± 4.00	85.20 ± 8.66	6.54 ± 0.12
	3rd week	5.01 ± 0.65	0.89 ± 0.25	9.04 ± 1.35	0.10 ± 0.00	143.90 ± 2.86^{a}	75.20 ± 4.42	77.00 ± 6.16^{b}	6.89 ± 0.09^{ab}
	4th week	7.39 ± 2.01	0.78 ± 0.17	9.00 ± 1.86^{a}	0.15 ± 0.05	140.79 ± 6.15^{a}	73.00 ± 3.00	62.20 ± 2.82^{b}	5.87 ± 0.29^{b}
	5th week	3.78 ± 0.27	0.68 ± 0.10	9.89 ± 2.11	0.10 ± 0.00	139.12 ± 7.07^{a}	72.40 ± 1.94	66.00 ± 4.15^{b}	6.39 ± 0.15
	7th week	6.89 ± 1.60	0.72 ± 0.15	9.96 ± 1.78	1.39 ± 1.02	146.19 ± 3.70^{a}	70.40 ± 1.17	79.80 ± 6.07	7.00 ± 0.15
	9th week	5.06 ± 0.82	0.84 ± 0.25	8.61 ± 2.17	1.45 ± 0.85	139.52 ± 6.17^{a}	67.20 ± 4.73	73.20 ± 8.44	6.55 ± 0.13
Gr-II (Post-pubertal Jaffrabadi heifers 48 ± 2 months; n=5)	1st week	4.35 ± 0.39	0.75 ± 0.29	6.99 ± 0.72^{b}	1.90 ± 1.13	143.19 ± 6.20^{a}	75.80 ± 3.12	71.80 ± 3.40	6.08 ± 0.31
	2nd week	4.38 ± 0.69	0.51 ± 0.09	7.76 ± 0.92^{b}	1.65 ± 1.54	141.53 ± 6.40^{a}	71.80 ± 4.33	83.20 ± 2.24	6.47 ± 0.23
	3rd week	4.07 ± 0.37	0.48 ± 0.06	7.75 ± 1.10	0.63 ± 0.45	144.29 ± 6.74^{a}	73.60 ± 1.69	70.60 ± 2.94^{b}	6.45 ± 0.13^{b}
	4th week	4.12 ± 0.60	0.64 ± 0.21	11.10 ± 3.42^{a}	2.01 ± 1.20	140.44 ± 7.82^{a}	75.00 ± 1.61	66.60 ± 3.28^{b}	6.03 ± 0.27^{b}
	5th week	4.53 ± 0.74	0.57 ± 0.08	8.99 ± 1.69	1.27 ± 0.80	150.71 ± 8.18^{a}	72.00 ± 4.64	79.80 ± 6.41^{ab}	6.76 ± 0.12
	7th week	4.05 ± 0.71	0.38 ± 0.06	7.45 ± 1.13	2.79 ± 1.17	144.79 ± 9.30^{a}	81.25 ± 5.15	73.50 ± 3.77	6.23 ± 0.32
	9th week	3.63 ± 0.26	0.67 ± 0.11	8.13 ± 1.18	1.28 ± 0.99	138.61 ± 9.14^{a}	73.75 ± 5.54	72.50 ± 3.66	6.48 ± 0.44
Gr-III (Post-partum lactating Jaffrabadi buffaloes; n=5)	1st week	7.19 ± 2.18	0.44 ± 0.04	4.00 ± 0.56^{c}	1.30 ± 0.73	58.52 ± 6.70^{b}	65.20 ± 4.77	98.20 ± 7.94	6.56 ± 0.43
	2nd week	6.71 ± 0.78	0.40 ± 0.07	4.33 ± 0.67^{c}	2.24 ± 0.62	57.12 ± 4.42^{b}	66.40 ± 4.11	102.60 ± 8.41	6.68 ± 0.30
	3rd week	7.23 ± 1.16	0.52 ± 0.07	4.75 ± 1.05	2.76 ± 1.17	64.79 ± 5.55^{b}	74.25 ± 2.69	106.00 ± 10.53^{a}	6.96 ± 0.11^{a}
	4th week	7.35 ± 1.26	0.59 ± 0.11	4.48 ± 0.88^{b}	0.71 ± 0.48	68.34 ± 6.56^{b}	69.75 ± 5.41	101.50 ± 9.67^{a}	6.81 ± 0.21^{a}
	5th week	6.66 ± 1.13	0.44 ± 0.05	6.39 ± 1.15	2.50 ± 0.67	59.11 ± 8.45^{b}	71.25 ± 1.25	96.75 ± 10.79^{a}	6.70 ± 0.37
	7th week	4.71 ± 1.03	0.42 ± 0.04	6.78 ± 1.04	0.74 ± 0.43	75.11 ± 6.97^{b}	70.00 ± 2.89	92.33 ± 12.99	6.64 ± 0.25
	9th week	7.14 ± 1.44	0.54 ± 0.06	6.23 ± 1.13	0.99 ± 0.52	64.61 ± 6.98^{b}	70.00 ± 0.82	95.00 ± 15.62	6.74 ± 0.33

Means bearing uncommon superscripts (a, b) differ significantly ($p < 0.05$) for a particular week between three groups.

true anestrus, and thus to initiate estrus synchronization protocols, embryo transfer treatments or transfer of embryos to recipients.

In the present study, irrespective of groups and periods, the plasma FSH concentrations ranged from 3.63±0.26 to 7.39±2.01 ng/ml, plasma LH concentrations from 0.38±0.06 to 0.89±0.25 ng/ml, plasma estrogen from 4.00±0.56 to 11.10±3.42 ng/ml, plasma progesterone from 0.10±0.00 to 2.79±1.17 ng/ml, plasma insulin from 57.12±4.42 to 150.71±8.18 pg/ml, blood glucose from 65.20±4.77 to 81.25±5.15 mg/dl, plasma cholesterol 62.20±2.82 to 106.00±10.53 mg/dl and plasma total protein levels 5.87±0.29 to 7.00±0.15 g/dl. The plasma levels of estrogen were significantly higher ($p < 0.05$) in Group-I as compared to Group-III and Group-II, which were at par, on first and second week of study period. However, plasma levels of estrogen increased significantly ($p < 0.05$) in Group-II and I as compared to Group-III on fourth week of study period. The mean plasma levels of insulin concentration in buffaloes of Group-III under study were significantly ($p < 0.01$) lower than in heifers of Group-I and Group-II throughout the study period (Table 2). The values of plasma cholesterol were significantly higher ($p < 0.05$) in Group-III as compared to Group-I and Group-II on third, fourth and fifth weeks of study period. The plasma total protein significantly increased ($p < 0.05$) in Group-III as compared to Group-II and I on third week of observation (Table 2). The comparison of the weekly data revealed that there were no significant differences in the plasma FSH, LH, progesterone and blood glucose levels in animals of three groups between periods or between groups on any of the sampling days.

Raval *et al.* (2020) reported almost similar pattern and range of plasma FSH, LH, estrogen, progesterone, insulin, blood glucose, plasma cholesterol and plasma total protein levels in double Ovsynch treated Jaffrabadi animals. However, the behavioural estrus was still not manifested by the present post-pubertal heifers till 50 months of age and lactating buffaloes till 5 months postpartum, though the ovarian changes and plasma endocrine profile revealed cyclicity with silent ovulations. These results are thus useful to diagnose anovulatory conditions and/or true anestrus and thereby initiate the estrus synchronization protocols for early conception in Jaffrabadi buffaloes.

In Jaffrabadi buffaloes and even in other breeds, we could not find any published report on ovarian follicular dynamics, hormonal and biochemical profile during the normal post-pubertal or postpartum period to co-relate our present findings. To the best of our knowledge and belief, this is perhaps the first study in Jaffrabadi buffaloes in their native tract.

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References

Danell, B. (1987). Oestrous behaviour, ovarian morphology and cyclical variation in follicular system and endocrine pattern in water buffalo heifers. *PhD thesis,* Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Devkota, B., Bohara, T.P., & Yamagishi, N. (2012). Seasonal variation of anestrus conditions in buffaloes (*Bubalus bubalis*) in Southern Nepal. *Asian Journal of Animal and Veterinary Advances, **7***(9), 910-914.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V.N., & Jain, G.C. (1997). Oocyte retrieval and histological studies of follicular population in buffalo ovaries. *Animal Reproduction Science, 47*, 189-195.

Malik, R.K. (2005). Studies on ovarian follicular dynamics during early postpartum period, anestrus condition and hormonal therapies for induction of estrus in Murrah buffaloes (*Bubalus bubalis*). *Ph.D. Thesis*, CCS Haryana Agricultural University, Hisar (India).

Mondal, S., Prakash, B.S., & Palta, P. (2007). Endocrine aspects of estrous cycle in buffaloes (*Bubalus bubalis*). *Asian-Australasian Journal of Animal Sciences, 20*(1), 124-131.

Pottapenjera, V., Rajanala, S.R., Reddy, C., Gangineni, A., Avula, K., Bejjanki, S.K., Sathagopam, S., Kesharwani, S., & Velmurugan, S. (2018). Kisspeptin modulates luteinizing hormone release and ovarian follicular dynamics in pre-pubertal and adult Murrah buffaloes. *Frontiers in Veterinary Sci*ences,*5*, 149.

Presicce, G.A., Bella, A., Terzano, G.M., De Santis, G., & Senatore, E.M. (2005). Postpartum ovarian follicular dynamics in primiparous and pluriparous Mediterranean Italian buffaloes (*Bubalus bubalis*). *Theriogenology, 63*(5), 1430-1439.

Presicce, G.A., Senatore, E.M., Bella, A., De Santis, G., Barile, V.L., De Mauro, G.J., & Parmeggiani, A. (2004). Ovarian follicular dynamics and hormonal profiles in heifer and mixed-parity Mediterranean Italian buffaloes (*Bubalus bubalis*) following an estrus synchronization protocol. *Theriogenology, 61*(7), 1343-1355.

Raval, R.J., Vala, K.B., Padodara, R.J., Dhami, A.J., & Kavani, F.S. (2020). Evaluation of double ovsynch protocol in acyclic Jaffrabadi heifers and buffaloes with respect to ovarian dynamics, hormonal profile and fertility. *Indian Journal of Animal Research*, 55: DOI, 10-18805/ijar.B-4154, First online published on 1-10-2020.

Snedecor, G.W., &Cochran, W.G. (1994). *Statistical Methods*. 8^{the}dn. Oxford & IBH Publishing Co., Calcutta, India.