RESEARCH ARTICLE

Interrelationships Between Ovarian Dynamics and Blood Plasma Profile in Double Ovsynch Treated Jaffrabadi Heifers and Buffaloes

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Abstract

A study was carried out on post-pubertal acyclic Jaffrabadi buffalo heifers (n = 12, age 40-50 months) and >90 days postpartum lactating acyclic buffaloes (n = 6) employing double Ovsynch protocol to evaluate the treatment response, ovarian dynamics, and hormonal and biochemical profile assessed on different days of treatment till day 35 post-FTAI and their correlations. The protocol consisted of i/m injections of 20 µg Buserelin acetate, a GnRH analogue on day 0, 10, 17 and 26, and 500 µg $PGF_2\alpha$, Cloprostenol, on day 7 and 24, with a timed insemination on day 27. The populations of small (4 mm), medium (4-8 mm) and large (>8 mm) size follicles, and the diameters of largest/ dominant and subordinate follicles did not vary between double Ovsynch treated acyclic heifers and buffaloes, but they were significantly ($p < 0.05$) interrelated among themselves in both the categories of animals. The overall mean plasma levels of LH, estrogen. insulin and conception rate were significantly ($p < 0.05$) higher and plasma FSH and total cholesterol were lower in treated heifers than in buffaloes. However, the levels of plasma FSH, LH, estrogen, progesterone and insulin concentrations did not reveal any appreciable correlations among themselves and with most of the follicular traits in buffaloes, but in heifers plasma LH had significant correlations with diameter of subordinate follicles and plasma total protein. Plasma estrogen was significantly ($p < 0.01$) correlated with blood glucose, protein and cholesterol in buffaloes, while progesterone showed significant negative correlations with blood glucose, total protein and most of the follicular traits in heifers. Plasma protein showed significant positive correlations with plasma insulin and cholesterol levels, and cholesterol with blood glucose, but their correlations with follicle population and diameter were negligible. It was concluded that higher conception rate in double Ovsynch treated acyclic heifers could be due to better synchrony of follicular events and plasma biochemical and endocrine constituents, culminating into ovulatory induced estrus compared to multiparous buffaloes.

Keywords: Biochemical profile, Correlations, Double Ovsynch, Hormonal profile, Jaffrabadi buffalo, Ovarian dynamics.

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Introduction

Anestrus is one of the most commonly encountered infertility problems in Indian buffaloes with the reported incidence of 20.84 to 45.97 % (Kulkarni *et al*., 2002; Modi *et al.,* 2011). The world famous Jaffrabadi breed of buffalo of Saurashtra region of Gujarat, India, is known for late maturity and poor reproductive efficiency. Ovarian dynamics and peripheral circulatory concentrations of various hormones 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 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 post-partum etc. (Mondal *et al*., 2007). To improve reproductive efficiency, estrus synchronization using "double Ovsynch protocol" gives better pre-synchrony of follicular waves before inducing CL is regressed for a timed AI breeding with subsequent better conception rates (Souza *et al.,* 2008; Dirandeh *et al.,* 2015; Stevenson, 2017; Raval *et al.,* 2020).

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However, the research work on this line in Jaffrabadi buffalo even in its native tract was meagre. Hence, the ovarian dynamics including follicle population and CL growth using trans-rectal USG as well as blood profiling for biochemical and endocrine constituents was studied just before each hormone treatment and then at fortnightly interval post-AI

and conception rate at FTAI in acyclic post-pubertal Jaffrabadi heifers and the postpartum lactating acyclic buffaloes employing double Ovsynch protocol on a well-managed organized dairy farm of the University in Junagadh, and the findings in respect of period-wise Mean ± SE values and conception rates have been reported earlier (Raval *et al.,* 2020). This paper was aimed to study and report the interrelationships between the above parameters of ovarian dynamics and blood biochemical and endocrine profile of same "double Ovsynch" treated acyclic Jaffrabadi buffalo heifers and multiparous buffaloes.

Materials and Methods

The study was carried out under tropical climate on 12 post-pubertal acyclic Jaffrabadi heifers (age 40-50 months) and six >90 days postpartum lactating acyclic buffaloes maintained under standard management practices at the University Farm, JAU, Junagadh, India, following approval of the Institutional Animal Ethics Committee. These animals were subjected to Double Ovsynch protocol (Dirandeh *et al.,* 2015, Raval *et al.,* 2020), which consisted of i/m injections of 20 µg Buserelin acetate, a GnRH analogue (Receptal, Intervet/MSD) on day 0, 10, 17 and 26, and 500 µg $PGF_2\alpha$ (Cloprostenol, Zydus Animal Healthcare) on day 7 and 24 with a timed insemination on day 27 at 16 and 24 hours of GnRH injection.

To monitor 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). All follicles with diameter <4 mm (small follicles), 4-8 mm (medium follicles) and >8 mm (large follicles) as well as CLs (Malik, 2005) were identified. Scanning of all animals was performed along with blood sampling in K_3EDTA vacutainers on each day of hormone injection and on day 0, 12, 21 and 35 post-AI, and plasma was stored at –20°C until analysed. Plasma concentrations of follicle stimulating hormone (FSH) (Cloud-Clone Corp., USA), luteinizing hormones (LH), estrogen and insulin (MyBio Source, 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 and blood profile were utilized to find out the Pearson's correlations (Snedecor and Cochran, 1994).

Results and Discussion

The overall mean values of various parameters including conception rates and Pearson's correlations found between the ovarian dynamics, *i.e.,* small, medium, large and total follicular population, diameters of largest and second largest (subordinate) follicles as well as plasma biochemical and endocrine profiles in Jaffarabai heifers (n = 12) and buffaloes (n = 6) over nine different periods of double Ovsynch protocol and their follow up till 35 days post-FTAI are presented in Table 1 and 2, respectively.

The average number of follicles of different size and the diameters of dominant and subordinate follicles did not vary significantly between post-pubertal heifers and multiparous buffaloes treated with Double Ovsynch protocol. However, the overall mean values of plasma FSH and total

Table 1: Overall mean (± SE) values of ovarian dynamics and blood plasma profile of post-pubertal Jaffrabadi heifers and buffaloes treated with Double Ovsynch protocol.

Parameters	*Jaffrabadi heifers*	*Jaffrabadi buffaloes*
No. of small follicles (< 4 mm)	6.62 ± 0.19	6.53 ± 0.23
No. of medium size follicles (4-8 mm)	4.11 ± 0.14	4.38 ± 0.16
No. of large size follicles (>8 mm)	2.99 ± 0.12	3.04 ± 0.22
Total No. of follicles	13.72 ± 0.37	13.96 ± 0.47
Diameter of large follicle (DLF, mm)	11.47 ± 0.26	12.47 ± 0.47
Dia. subordinate follicle (DSF, mm)	9.73 ± 0.23	10.26 ± 0.39
Plasma FSH (ng/ml)**	6.89 ± 0.29	10.00 ± 0.54
Plasma LH (ng/ml)**	0.79 ± 0.05	0.38 ± 0.01
Plasma Estrogen (ng/ml)*	10.76 ± 0.89	7.36 ± 0.38
Plasma Progesterone (ng/ml)	1.82 ± 0.21	2.03 ± 0.29
Plasma Insulin (pg/ml)**	144.34 ± 1.40	78.05 ± 3.94
Blood Glucose (mg/dl)	71.98 ± 0.80	70.76 ± 1.15
Plasma Total cholesterol (mg/dl)*	75.99 ± 1.71	90.49 ± 4.85
Plasma Total protein (mg/dl)	6.57 ± 0.05	6.95 ± 0.04
Conception rate at induced estrus (%)*	75.00	16.66

*P < 0.05, **P < 0.01 between buffalo and heifers.

Table 2: Correlation matrix (r) of ovarian dynamics and plasma hormonal and biochemical profiles in Jaffrabadi buffaloes treated with double Ovsynch protocol

Parameters	*SF*	*MF*	*LF*	*TF*	*DLF*	*DSF*	*FSH*	*LH*	*E2*	*P4*	*Insulin*	*Glucose*	*TC*	*TP*
Correlations - Post-pubertal Heifers														
No. of small follicle (SF)	1	0.36**	0.42**	0.83**	0.39**	0.46**	0.10	0.17	-0.04	-0.18	0.04	0.23*	-0.28**	-0.21*
No. of medium follicle (MF)	0.09	1	0.31**	0.72**	0.05	0.09	0.05	0.05	0.22*	-0.16	0.24*	0.15	-0.21*	-0.12
No. of large follicle (LF)	0.28	0.07	1	0.72**	0.45**	0.63**	0.19	0.14	-0.01	-0.21*	0.18	0.18	-0.03	-0.16
Total No. of follicles (TF)	0.71**	0.44**	0.78**	1	0.40**	0.51**	0.14	0.16	0.07	-0.22*	0.19	0.25*	-0.25*	-0.22*
Diameter, large follicle (DLF)	0.33*	0.08	0.66**	0.60**	1	0.74**	-0.04	0.14	-0.05	-0.24*	0.26*	0.10	-0.10	-0.13
Dia. subordinate follicle (DSF)	0.27	-0.12	0.75**	0.56**	0.76**	1	-0.07	0.28**	-0.06	-0.25*	0.22*	0.12	-0.17	-0.23*
Plasma FSH	0.11	-0.08	-0.20	-0.10	-0.17	-0.18	1	0.07	0.06	-0.10	0.10	-0.07	0.06	0.07
Plasma LH	-0.07	-0.17	0.01	-0.09	-0.12	0.11	0.08	1	-0.08	-0.10	0.07	-0.16	-0.03	-0.29**
Plasma Estrogen (E2)	-0.27	-0.19	0.07	-0.16	-0.06	0.20	0.06	0.23	1	0.02	0.13	-0.17	-0.43**	0.09
Plasma Progesterone (P4)	0.18	0.09	0.08	0.16	0.10	0.09	0.12	0.06	0.11	1	0.04	-0.28**	-0.04	0.21*
Plasma Insulin	0.16	0.07	-0.09	0.05	-0.26	-0.10	0.02	0.06	0.24	0.01	1	0.08	-0.08	0.01
Blood Glucose	-0.17	-0.14	0.16	-0.03	0.06	0.17	-0.21	-0.02	0.45**	0.10	-0.12	1	-0.30**	-0.18
Plasma Total cholesterol (TC)	-0.25	-0.18	0.19	-0.08	0.21	0.33*	-0.12	0.08	0.73**	0.12	-0.07	0.52**	1	0.42**
Plasma Total protein (TP)	-0.08	0.01	-0.16	-0.14	-0.26	-0.02	0.06	0.15	0.58**	0.05	0.35*	0.09	0.47**	1
Correlations - Multiparous Buffaloes														

*Significant at the p < 0.05 level; **Significant at the p < 0.01 level (2-tailed); n = heifer 108 observations, buffalo 54 observations.

cholesterol were significantly (p < 0.05) higher, while plasma LH, estrogen, insulin and conception rate were significantly lower in buffaloes than in heifers, and the values of plasma progesterone, glucose and total protein were statistically similar among them (Table 1). Similar trend was also noted for period-wise values in these animals (Raval *et al.,* 2020). Efficiency of ovulation synchronization protocol depends on the ovarian response to the first GnRH injection, and it is recognized as an important factor in synchronization of the ovulations for FTAI (De Jarnette *et al.,* 2001). The mean diameters of large follicles in the present study were also close to the previous report of Jerome *et al.* (2016). Higher conception rate in post-pubertal heifers may be correlated to the fact that primiparous cows are more likely to be anovular compared with multiparous and respond well to hormone therapy (Silva *et al.,* 2007). Dirandeh *et al.* (2015) also reported increased pregnancy per AI with double Ovsynch in primiparous (65.2 vs 45.2%) as compared to multiparous (37.5 vs 39.3%) cows.

Correlations of Ovarian Dynamics Parameters

In post-pubertal Jaffrabadi heifers treated with double Ovsynch protocol, the small follicle population showed significant (p < 0.01) positive correlations with medium, large and total number of follicles (r = 0.36, 0.42, 0.83), diameter of large and subordinate follicles and blood glucose (r = 0.39, 0.46, 0.23), and negative correlations with plasma levels of total cholesterol and protein (-0.28, -0.21). The medium size follicle population was significantly correlated with large follicle population and total number of follicles (0.31, 0.72, p < 0.01) as well as with plasma estrogen, insulin (0.22, 0.24; p < 0.05) and plasma cholesterol (-0.21). However, large size follicle population revealed highly significant positive correlations with total follicle population as well as diameters of largest and subordinate follicles (0.72, 0.45, 0.63, p < 0.01), and had non-significant high correlations with glucose and plasma insulin concentrations (0.18 each) and negative correlation with plasma progesterone (-0.21). Further, the total number of follicles in heifers were highly significantly correlated with diameters of largest and subordinate follicles (0.40, 0.51), blood glucose, insulin (0.25, 0.19), and negatively with plasma progesterone, cholesterol and protein (-0.22, -0.25, -0.22, p < 0.05). The diameters of largest and subordinate follicles were significantly interrelated (0.74) and both were positively correlated with plasma LH (0.14, 0.28), insulin (0.26. 0.22), and negatively with plasma progesterone (-0.24, -0.25) and protein (-0.13, - 0.23) (Table 2).

However, among postpartum lactating buffaloes, the small follicle population had significant (p < 0.01) positive correlations only with total number of follicles and diameter of largest follicle (0.71, 0.33). The medium size follicle population was positively correlated only with total number of follicles (0.44), but not with other traits. Large follicle population showed highly significant positive association with total

number of follicles (0.78) as well as diameters of largest and subordinate follicles (0.66, 0.75), but not with plasma constituents studied. Similarly, the total number of follicles was significantly correlated only with diameters of largest and subordinate follicles (0.60, 0.56), and later diameters in turn were significantly and positively interrelated (0.76), but none of them showed significant association with plasma biochemical or hormonal constituents studied.

Correlations of Blood Plasma Constituents:

In heifers, the plasma FSH did not reveal significant correlations with any of the ovarian or plasma traits, while the plasma LH concentrations showed positive correlation with diameter of subordinate follicles (0.28) and negative correlation with plasma protein (-0.29). Plasma estrogen levels had positive correlation with medium size follicle population (0.22) and negative correlation only with plasma cholesterol (-0.43). Plasma progesterone had significant negative correlations with most of the follicular traits (-0.16 to -0.25, $p < 0.05$) and glucose (-0.28), and positive correlation with total protein (0.21), while in multiparous buffaloes all these correlations were negligible, except of estrogen with glucose, cholesterol and protein (0.45, 0.73, 0.58, $p < 0.01$). Plasma insulin concentration showed significant positive correlations with ovarian follicular traits only in heifers (0.18 to 0.26), but not in buffaloes. The total cholesterol had significant negative correlation with blood glucose (-0.30) and positive correlation with plasma protein (0.42) in heifers, while in buffaloes the total cholesterol had significant positive correlations with diameters of subordinate follicles, plasma estrogen and glucose (0.33, 0.73, 0.52), and total protein with plasma estrogen, insulin and cholesterol (0.58, 0.35, 0.47). In short the correlations of ovarian follicular traits with plasma biochemical and endocrine constituents studied were negligible particularly in lactating buffaloes treated with double ovsynch protocol (Table 2).

The correlations found were physiological and as per expectations. Kumar (2015) found positive correlation between pre-ovulatory follicular diameter and plasma estradiol ($r = 0.84$, $p < 0.05$) on the day of estrus in buffaloes, and higher plasma estradiol on the day of estrus was also positively associated with conception rate. Hegazy *et al.* (1994) reported significant negative correlation ($r = 0.5$, $p < 0.05$) between serum glucose concentration during first eight week postpartum and the interval to first ovulation and first detected estrus. Significantly lower glucose has been reported responsible for pubertal and postpartum anestrus, indicating its positive association with fertility (Rahbar *et al.,* 2014; Dhami *et al.,* 2017; Vala *et al.,* 2020). Optimum protein level is necessary for the development of endocrine and sex organs. Insulin stimulates the release of GnRH from hypothalamus and LH from pituitary (Tanaka *et al*., 2000). Insulin regulates CL function by increasing glucose availability and thus production of hormone in cow (Sousa *et al*., 2016). In addition, insulin may also act on the pituitary gland to increase gonadotroph sensitivity to GnRH (Solorzano *et al*., 2010). The literature reviewed however did not reveal comparable correlation studies with synchronization protocols in dairy animals to further discuss the present correlation findings. Moreover, very few studies were available in the literature even on use of double Ovsynch protocol in bovines and buffaloes in particular, suggesting need to undertake further studies in this protocol to prove its efficacy and worth in anestrus buffaloes.

From the findings, it can be concluded that higher conception rate in double Ovsynch treated acyclic heifers could be due to better synchrony of follicular events and plasma biochemical and endocrine constituents, culminating into ovulatory induced estrus compared to multiparous buffaloes.

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