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INFLUENCE OF SEASONAL NUTRITION WITH OR WITHOUT SUPPLEMENTATION ON THE METABOLIC STATUS AS WELL AS THE OVARIAN RESPONSE AND EMBRYO PRODUCTION IN SUPEROVULATED BUFFALO-COWS

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SUMMARY

A total number of 40 non lactating pluriparious buffalo-cows were randomly allocated into 5 groups. During both winter and spring seasons, 22 buffalo-cows were allocated into 2 experimental groups. Group I (n= 15) was fed on about 25 kg of Berseem + 2 kg of wheat straw, while Group II (n=7) was fed on 25 kg of berseem + 3 kg of concentrate mixture. During the next season (summer), another group of buffalo-cows (Group III, n=7) were fed on 5 kg of the concentrate mixture + 3 kg of wheat straw. At autumn, 11 buffalo-cows were allocated into two groups. Group IV (n=5) were fed on about 25 kg green corn + 2 kg of wheat straw, while the last group (Group V, n=6) was fed on 25 kg of green corn + 3 kg of the concentrate mixture. The seasonal nutrition had a highly significant effect ($P<0.001$) on serum progesterone, estradiol-17β, cortisol, glucose, cholesterol, calcium and copper as well as a significant ($p<0.01$) effect on the serum albumin and phosphorous. The response to superovulation were 80%, 100% , 71.4% , 80.0% and 83.3% in group I, II, III, IV and V, respectively. Changes in the dietary intake from berseem only to berseem supplemented with concentrate was associated with a significant ($P<0.05$) improvement in the number of ovulation (4.8±0.49 VS 5.86±0.51), recovered embryo/ova (3.08±0.4 VS 3.86±0.40), fertilized ova (2.58±1.78 VS 3.43±0.37) and the number of grade I transferable embryos (1.58±0.19 VS 2.57±0.30). Comparison between group IV and V revealed that, Supplementation of concentrate to green corn also improved the number of ovulation (4.4±0.68 VS 5.5±0.76), collected embryo/ova (2.75±0.29 VS 3.6±0.27) and transferable embryos of grade I (1.25±0.25 VS 2.2±0.20). In general, no significant differences were observed between group II

and group V on one hand or between group I, II and IV on the other hand. Positive correlation coefficient were noted between progesterone concentration at the day of embryo collection and ovulation rate (r=0.91; P<0.001), recovered embryo/ova (r=0.70; P<0.001), fertilized ova (r=0.66; P<0.001) and Grade I transferable embryo (r=0.37; P<0.05). Also between progesterone prior to hormonal treatment and number of CL (r=0.70; P<0.001) and fertilized ova (r= -0.49; P<0.01). A positive correlation coefficient (r =0.31; P<0.01) was noted between estradiol-17β concentration before treatment and number of follicles in the superovulated buffaloes as well as at the day of recovery and follicles (r=0.87; P<0.001) and Grade II transferable embryos (r=0.31; P<0.01). Negative correlation coefficient was observed between cortisol concentration before treatment and fertilized ova (r= 0.32; P<0.05) and with grade II transferable embryos (r= - 0.40; P<0.01). At the same time, a positive correlation were noted between serum glucose before treatment (r=0.71; P<0.01), at the day of collection (r=0.51; P<0.05) and the fertilization rate (r=0.51; P<0.05). Also a positive correlation was found between total cholesterol level at the day of embryo recovery and fertilized ova (r=0.41; P<0.05).

INTRODUCTION

Although water buffaloes are among the most important domestic ruminants providing milk in over 40 countries, only 0.1% of their population produce 3500-4000 kg milk in 305 day of lactation (Misra et al., 1990). The techniques of superovulation and embryo transfer coupled with progeny testing can play an important role in the propagation of these precious sources of germplasm (Taneja et al., 1994). However, variability of superovulation response and poor embryo recovery are the major limitations in the commercial exploitation of embryo transfer technology. There are many external factors which influence the superovulatory response and the yield of transferable embryos in donor animals. Among these, season and nutrition can play important roles (Dunn, 1980). However, the effect of temperature and humidity during different seasons are fixed and less likely to be manipulated by management. At the same time the variation in the season of flushing which accompanied with changes in the nutrition has been affected the number of viable embryos recovered from superovulated cattle (Brown et al., 1991) so, they should be considered when devising management strategies. On the other hand, the majority of buffaloes in Egypt are owned by farmers (small holders), most of them feed their buffaloes according to their own experience and the available fodder in different seasons of the year (EL-Ashry, 1988). Hence, deficient nutrient intake is a common problem especially when feed supplementation (grains, cakes or other concentrates) is frequently absent.

Nutrition alone involve a series of components that can affect superovulation and embryo quality in different ways. Plane of nutrition could affect the response of beef cattle to exogenous FSH (Staigmiller et al., 1979). Lucy et al. (1991) reported that increasing energy balance is proportional to the number of large follicles in postpartum dairy cows. Meanwhile, negative energy balance reduce response to estrous synchronization, and superovulation rate in cattle (Roberson et al., 1992). Dietary crude protein and its types (degradable & undegradable) could affect fertilization and embryo quality in dairy cows (Blanchard et al., 1990). On the other side, alteration in dietary lipid could potentially increase the number of follicles available to response to gonadotropins treatment in superovulation regimens (Ryan et al., 1992). It was though that, the metabolic conditions of buffaloes is closely correlated to reproductive efficiency including ovarian response to hormonal stimulation (Kweon et al., 1986).

The present study aimed to clarify the effect of seasonal nutrition with or without feed supplementation on ovarian response and embryo production in superovulated buffalo-cows, as well as to substantiate more concretely, the relationship between the metabolic status (prior to-and during-superovulation and at the day of embryo recovery) and the subsequent ovarian response with embryo production.

MATERIALS AND METHODS

Animals, feeding and experimental design:-

A total number of 40 non lactating pluriparious buffalo-cows (Bubalus bubalis), 4 -8 years of age, weighting 490-600 kg and having regular estrous cycle were used in this study. The experiment was applied at the experimental farm of Animal Reproduction Research Institute over 4 concessive seasons (Winter, Spring, Summer and Autumn). Animals feeding was depending on the available forages in different seasons with or without concentrate supplementation (as represented in table 1).

From the start of winter until the end of spring season, 22 buffalo-cows were divided into 2 experimental groups. Group I (n= 15) were fed on about 25 kg of Egyptian clover (Berseem) plus 2 kg of wheat straw, while Group II (n=7) was fed on 25 kg of berseem plus 3 kg of the concentrate mixture. During the next season (summer), another group of buffalo-cows (Group III, n=7) were fed on 5 kg of the concentrate mixture plus 3 kg of wheat straw. At autumn, another 11 buffalo-cows were allocated into two groups. Group IV (n=5) were fed on about 25 kg green corn (Darrhwa) with 2 kg of wheat straw, while the last group (Group V, n =6) was fed on 25 kg of green corn with 3 kg of the concentrate mixture. Composition and chemical analysis of the used diets are shown in table 2.

Table 1. Feeding regimes of the experimental groups during different seasons

Season (s)	group	No. of animals	feeding
Winter + Spring	I	15	25 kg berseem + 2 kg wheat straw
Winter + Spring	II	7	25 kg berseem + 3 kg concentrate mixture
Summer	III	7	5 kg concentrate mixture + 3 kg wheat straw
Autumn	IV	5	25 kg darrhwa + 2 kg wheat straw
Autumn	V	6	25 kg darrhwa + 3 kg concentrate mixture

Table 2. Composition and chemical analysis* (on dry matter basis) of the used diets

Ingredients	DM %	C.P%	TDN %**	EE %	Ca %	P %
Concentrate mixture ***	92.6	13.1	61.2	2.70	1.44	0.70
Egyptian clover (Berseem)	15.0	17.3	61.0	3.30	1.66	0.53
Green corn (Darrhwa)	22.5	7.4	58.70	1.07	0.40	0.13
Wheat straw	91.7	4.03	44.70	0.02	0.18	0.05

DM=dry matter, CP=crude protein, TDN=total digestible nutrients, EE= ether extract, Ca= calcium and P= phosphorus

* Analyzed according to AOAC (1980). ** Estimated according to Haris et al.,(1972)

*** Consists of 30% wheat bran, 30% yellow corn, 13.5 % decortecated cotton seed meal (41% cp), 18.4% berseem straw, 5% molasses, 2% lime stone and 1% common salt.

Superovulation and embryo recovery:-

Estrous cycle of the selected animals were synchronized by a single dose of 5 ml luteolytic hormone (Lutalyse, Upjohn Co., Belgium) when a well developed and clear CL could be palpated. Double doses, 11 days apart were used when CL was not clear. After luteolytic hormone injection, the animals were carefully observed visually twice daily for estrous behavior. After synchronization, a single dose of 3000 IU of pregnant mare serum gonadotropin (PMSG/Folligon; Intervet Co., B.V. Holland) was injected IM on day 10-12 of the estrus. Two days later, 2 doses of luteolytic hormone were injected with 12 hr. interval. The animals were naturally mated by a fertile bull during estrous phase. On the day of embryo collection (Day 6, estrus=Day 0), efficacy of superovulation was determined by estimated the No. of ovulation (palpable CL) in each animal and counting the unovulated follicles > 10 mm per rectum. Recovery of embryos was performed non surgically, using method described by NewComb et al (1978). Morphological evaluation has been used to delineate embryo quality, the evaluation was applied according to Linder and Wright (1983) and Takeda (1986). The transferable embryos were classified as grade I (Excellent and good) and grade II (Fair and poor).

Hormonal and biochemical assays:-

Peripheral blood samples were collected from all buffaloes before superovulation for 3 successive days. During superovulatory regime, one sample was collected every second day then one sample was taken at day of embryo recovery. The serum was separated, where serum glucose levels were measured within 4 hr.. The rest serum was stored at - 20 °C for other blood parameters.

The collected sera of all animals were used for estimation of progesterone, estradiol-17β and cortisol hormones by using radioimmunoassay (RIA). Progesterone concentration was determined according to Abraham (1981). Intra-and inter-assay coefficient of variations were 9.5 and 13%, respectively. Estradiol-17β was also estimated according to Xing et al. (1983). Intra- and inter- assay coefficient of variations were 7 and 8.1%, respectively. Cortisol was analyzed using the method of Foster and Dunn (1974). The intra- and inter- assay coefficient of variations were 6.3 and 9.6%, respectively.

All serum samples were enzymatically analyzed for glucose, total protein, albumin (Kits of Sclavo Inc., Italy). Globulin was estimated by difference between total protein and albumin. Cholesterol, calcium and inorganic phosphorous (kits of Bio-Merieux, France). Serum zinc and copper were measured using atomic absorption spectrophotometer (Perkin Elmer, Mod. 3300, USA).

Statistical analysis:-

All data were statistically analyzed using PCSTAT computer program and according to Snedecor and Cochran (1980). Data of blood parameters were analyzed by two way classification analysis of variance technique in which the main effects of treatment groups (seasonal nutrition) and time of sampling and their interactions were examined. The obtained results of blood samples that were taken before superovulation were averaged and introduced into analysis as one sample. One way analysis of variance was used to analyzed the effect of seasonal nutrition on ovarian response and embryo production. Simple correlation coefficients were performed between ovarian response and embryo quality and each of blood parameters before treatment or at the day of embryo recovery.

RESULTS

The daily nutrient intake of buffalo-cows during different seasonal nutrition in relation to their daily requirements are presented in table 3. During winter and spring, feeding of berseem alone (group I) was associated with suboptimal levels of energy (87.8% TDN from the requirement). While addition of concentrate mixture to berseem (group II) during these seasons correct this deficient (102.8% for TDN from the requirement). On the other hand, during summer (group III), feeding of dry diet (concentrate + straw) covered the requirement of energy, protein, Ca and phosphorus. Meanwhile, during autumn season (group IV) feeding of Darrhwa alone led to suboptimal levels of both crude protein (87.5% from the requirement) and phosphorus (47.30% from the requirement). Addition of concentrate mixture (group V) rebalanc the concentrations of those two parameters (155.9% and 175.0% for CP and phosphorus, respectively).

The effect of seasonal nutrition and time of sampling on the hormonal as well as metabolic status in superovulated buffalo-cows is shown in tables 4,5 and 6. ANOVA (Table 4) revealed that, the season of nutrition had a highly significant effect ($P<0.001$) on the serum progesterone, estradiol-17β, cortisol, glucose, cholesterol, calcium and copper as well as a significant ($P<0.01$) effect on the serum albumin and phosphorous. However, the effect of seasonal nutrition on serum total protein, globulin and zinc were non significant. At the same time, the effect of sampling time on the blood parameters was highly significant ($P<0.01$-0.001) for progesterone, cortisol, estradiol-17β, glucose and tended to be significant ($P<0.06$-0.09) in cholesterol, and calcium. While, it had a non significant effect up on the other parameters (total protein, albumin, globulin, P, copper, and zinc). Meanwhile, the interaction between seasonal nutritional and time of blood sampling were significant on cortisol ($P<0.001$), glucose, cholesterol, zinc ($P<0.01$) and phosphorous ($P<0.6$-0.09).

As shown in table (5), blood analysis of buffalo-cows in group II revealed significantly ($P<0.05$) higher concentrations of progesterone, estradiol-17β, glucose and phosphorous than those in group I. However, the levels of cortisol was significantly ($P<0.05$) lower in group II than those in group

Table 3: Daily nutrient intakes of buffalo-cows in different experimental groups in relation to their requirements.

Nutrient	Requirement*	Group I	Group II	Group III	Group IV	Group V
TDN	4.0 kg	3.51(87.8%**)	4.11(102.8%)	4.29(107.3%)	4.53(113.3%)	5.13(128.3%)
CP	412.5 kg	761(184.5%)	1043(252.8%)	766(185.7%)	361(87.5%)	643(155.9%)
Fat	g***	125	206	136	6.6	87
Ca	21.0 g	62.0(295.2%)	105.0(500%)	72.0(342.8%)	22.5(107.1%)	65.7(312.9%)
P	16.0 g	20.0(125.0%)	41.0(256.3%)	35.0(218.8%)	7.52(47.30%)	28.0(175.0%)

*Daily nutrient requirement (maintenance) of buffalo- cows (weighting 550 kg) according to Ranjhan and Pathak (1979).

**Values in parenthesis were % from the requirement.

*** no specific recomendation.

Table 4: ANOVA summary of the effect of seasonal nutrition (S) and time of sampling (T) on the hormonal and metabolic status of superovulated buffalo-cows:-

Item	Main effects test of significance		
	Seasonal nutrition (S)	Time of sampling (T)	Interaction (S x T)
Progesterone	***	***	NS
Esradiol-17β	***	**	NS
Cortisol	***	***	***
Glucose	***	**	**
Total protein	NS	NS	NS
Albumin	**	NS	NS
Globulin	NS	NS	NS
Cholesterol	***	*	**
Calcium	***	*	NS
Phosphorus	**	NS	*
Copper	***	NS	NS
Zinc	NS	NS	**

NS = Non significant * P < 0.06 - 0.09. ** P < 0.01. *** P < 0.001.

Table 5: Effect of seasonal nutritional with or without concentrate supplementation on the hormonal and metabolic status of superovulated buffalo-cows (Mean ±SE):

parameters	Group I	Group II	Group III	Group IV	Group V
Progesterone (ng/ml)	3.31±0.35b	4.31±0.65 a	2.86 ±0.27 c	3.20±0.49 b	3.49±0.51 b
Estradiol 17 B (pg/ml)	3.39±0.43 b	4.99±0.78 a	3.05±0.37 bc	3.39±0.43 b	2.20±0.41 c
Cortisol (ng/ml)	7.47±0.68 c	6.95±0.76 d	8.94±1.12 a	8.40±0.95 b	7.94±0.69 b
Glucose (mg/dl)	46.09±2.07 b	64.56±3.55 a	46.91±3.33 b	52.35±4.04 b	71.65±8.30 a
Total protein (gm/dl)	7.52±0.20 a	7.50±0.40 a	7.69±0.36 a	7.39±0.30 a	7.38±0.19 a
Albumin (g/dl)	3.71±0.14 b	3.5±0.16 bc	3.38±0.17 c	3.75±0.23 b	4.30±0.12 a
Globulin (gm/dl)	3.95±0.09 a	4.00±0.38 a	4.33±0.40 a	3.69±0.31 a	3.11±0.24 a
Cholesterol (mg/dl)	54.84±1.99 a	55.03±4.99 a	51.44±2.41 ab	37.68±2.32 c	49.19±1.67 b
Calcium (mg/dl)	9.68±0.11 a	9.75±0.16 a	9.54±0.21 a	8.08±0.25 b	9.44±0.16 a
Phosphorous (mg/dl)	4.74±0.15 ad	5.62±0.29 c	5.22±0.14 b	4.62±0.21 d	5.02±0.15 ab
Copper (mg/L)	0.50±0.019 a	0.53±0.028 a	0.70±0.033 c	0.64±0.036 b	0.64±0.025 b
Zinc (mg/L)	0.45±0.019 a	0.48±0.018 a	0.45±0.022 a	0.48±0.031 a	0.45±0.012 a

Means in the same row with different superscripts differ significantly (P<0.05).

I. When compared to group IV, buffalo-cows in group V had significantly (P<0.05) higher concentrations of glucose, albumin, cholesterol, calcium and phosphorus. Meanwhile, Buffalo-cows fed on concentrate mixture+straw (group III) had significantly (P<0.05) higher concentrations of cortisol and copper than the other groups.

Table (6) illustrated the relationship between blood parameters and time of sampling related to superovulation treatment. Amongst all treated buffalo-cows in the present investigation, the concentrations of serum hormones (progesterone, estradiol-17β and cortisol) and glucose were significantly (P<0.05) higher in samples taken at day of recover than those taken before or during superovulation. Cholesterol was significantly (P<0.05) higher in samples of day of recovery than those during superovulation. Calcium was also significantly (P<0.05) higher at day of recovery and during superovulation than before that. On the other hand, serum total protein, albumin, phosphorous, copper and zinc were non significantly affected by sampling time.

The efficacy of ovarian response and embryo production of the treated buffaloes during different seasons of nutrition are shown in table (7). A percentage of 80% of buffalo-cows in group I and 100% of group II were responded to superovula-

Table6: Influence of time of sampling on the hormonal and metabolic status of superovulated buffalo-cows (mean ±SE):-

parameters	Sampling time		
	Before superovulation	During superovulation	Day of recovery
Progesterone (ng/ml)	2.30±0.25a	2.36±01.9 a	4.97±0.47 b
Estradiol 17 B (pg/ml)	2.28±0.24 a	3.09±0.32 b	4.94±0.52 c
Cortisol (ng/ml)	4.32±0.18 a	6.53±0.20 b	12..90±0.32 c
Glucose (mg/dl)	52.22±2.87 a	52.50±2.93 a	67.63±7.25 b
Total protein (gm/dl)	7.38±0.22 a	7.55±0.23 a	7.67±0.26 a
Albumin (gm/dl)	3.68±0.14 a	3.58±0.15 a	3.80±0.16 a
Globulin (gm/dl)	3.65±0.28 a	4.00±0.28 a	4.03±0.37 a
Cholesterol (mg/dl)	50.77±2.10 a	45.98±2.53 b	51.91±3.87 a
Calcium (mg/dl)	9.08±0.2 4 a	9.44±0.13 b	9.29±0.20 ab
Phosphorous (mg/dl)	5.13±0.16 a	5.06±0.13 a	4.91±0.14 a
Copper (mg/L)	0.61±0.029 a	0.62±0.029 a	0.56±0.027 a
Zinc (mg/L)	0.48±0.012 a	0.49±0.013 a	0.46±0.023 a

Means in the same row with different superscripts differ significantly (P<0.05).

tion by developing more than 2 CL (difference between them tended to be significant at P<0.1), while, only 71% of animals of group III responded to superovulation. The response was 80.0% and 83.3% in group IV and V, respectively (without significant difference). Changes in the dietary intake from berseem only to berseem supplemented with concentrate was associated with a significant (P<0.05) improvement in the number of ovulation (4.8±0.49 VS 5.86±0.51), recovered embryo/ova (3.08±0.40 VS 3.86±0.40), fertilized ova (2.58±1.78 VS 3.43±0.37) and the number of grade I transferable embryos (1.58±0.19 VS 2.57±0.30). Comparison between group IV and V, supplementation of concentrate to green corn improved the number of ovulation (4.4±0.68 VS 5.5±0.76), collected embryo/ova (2.75±0.29 VS 3.6±0.27) and transferable embryos of grade I (1.25±0.25 VS 2.2±0.20). In general, no significant differences were observed between group II and group V on one hand or between group I, III and IV on the other hand. The ovulation rates , rate of fertilization and the number of transferable embryos (Grade I) in our investigation were higher (P<0.05) in winter and spring seasons (5.86±0.51, 3.43±0.37 and 2.57±0.30, respectively in group II supplemented with concentrate) than summer season (4.29±0.78, 2.80±0.20 and 1.40±0.24, respectively in group III).

Across all treated buffalo-cows in this investigation, the correlation coefficient (untabulated data) between hormonal and metabolic profile and ovarian response and embryo quality revealed that, a positive correlation coefficient were noted between progesterone concentration at the day of embryo collection and ovulation rate (r=0.91; P<0.001), recovered embryo/ova (r=0.70;

Table 7: Effect of seasonal nutritional with or without concentrate supplementation on superovulation and embryo production in buffalo-cows (Mean ±SE):

Item	Group I	Group II	Group III	Group IV	Group V
No. of treated animals	15	7	7	5	6
Superovulation response*	12 (80%) a	7 (100%) b	5 (71.4%) a	4 (80.0%) a	5 (83.3%) ab
Corpus luteum	4.80±0.49 b	5.86±0.51 a	4.29±0.78 b	4.40±0.68 b	5.50±0.76 ab
Follicles >10 mm	2.07±0.33 bc	1.14±0.34 a	1.67±0.42 ab	1.25±0.50 a	2.6±0.40 c
No. of embryos & ova	3.08±0.40 bc	3.86±0.40 a	3.60±0.27 ab	2.75±0.29 c	3.60±0.27 ab
No. of fertilized ova	2.58±1.78 b	3.43±0.37 a	2.80±0.20 b	2.50±0.29 b	3.00±0.45 ab
Transferable embryos:					
Grade I	1.58±0.19 b	2.57±0.30 a	1.40±0.24 b	1.25±0.25 b	2.20±0.20 a
Grade II	1.00±0.17 a	0.71±0.29 a	0.80±0.37 a	1.00±0.41 a	0.80±0.37 a

Means in the same row with different superscripts differ significantly (P<0.05).
* >2 CL.

P<0.001), fertilized ova (r=0.66; P<0.001) and Grade I transferable embryo (r=0.37; P<0.05). Also between progesterone prior to hormonal treatment and number of CL (r=0.70; P<0.001) and fertilized ova (r=0.49; P<0.01). Positive correlation coefficient (r=0.31; P<0.01) was noted between estradiol-17β concentration before treatment and number of follicles in the superovulated buffaloes as well as between estradiol concentration at the day of recovery and no of follicles (r=0.87; P<0.001) and Grade II of transferable embryos (r=0.31; P<0.01). Negative correlation coefficient was observed between cortisol concentration before treatment and fertilized ova (r=-0.32; P<0.05) and with grade II transferable embryos (r= -0.40; P<0.01). At the same time, a positive correlation were noted between serum glucose before treatment (r=0.71; P<0.01), at the day of collection (r=0.51; P<0.05) and the fertilization rate. In the current study, it was noted that, those buffalo cows possess a high concentration of total cholesterol had a high numbers of CL, follicles, recovered embryos and Grade I transferable embryos, as well as positive correlation was found between total cholesterol level at the day of embryo recovery and fertilized ova (r=0.41; P<0.05).

DISCUSSION

The higher concentrations of both progesterone (P4) and estradiol-17β (E2) in buffalo-cows of group II than those in group I may related to the high fat content of the diet in group II. Similarly, Talavera et al.(1985) and Hawkins et al.(1985) reported an increase in circulating P4 in cows supplemented with fats. Such increase may attributed to a decrease in P4 clearance rate and not to an increase in P4 secretion by the corpus luteum (Hawkins et al., 1985). Also, Lammoglia et al. (1997) noted an increase in E2 concentrations in fat treated does (female goat) than control, although granulosa culture E2 production did not differ between treatments. They suggested that the clearance rate of E2 as previously shown for P4 was affected by dietary fat. Meanwhile, the significant increase of glucose and phosphorus in group II comparing to group I may attributed to the high energy (TDN) and phosphorus content of the diet of this group. The elevated concentration glucose, albumin, cholesterol, calcium and phosphorus in group V than those in group IV may also due to the high energy, protein, fat, calcium and phosphorus content of the diet of group V. On the other hand, Gmbe and Hansel (1973) found that, low nutrition acts to decrease ovarian progesterone synthesis and reduces feedback inhibition of gonadotropin at the hypothalamic-pituitary level. Moreover, plane of nutrition could affect the response of beef cattle to exogenous FSH (Staigmiller et al., 1979). At the same time, Buffalo cows fed on concentrate mixture+straw (group III) had significantly (P<0.05) higher concentrations of cortisol and copper than the other groups. In spite of the nutrient requirements of this group were covered, it had a significantly (P<0.05) lower level of progesterone. Such effect may attributed to the summer stress. Similar results were reported by Berman (1991) who recorded lower level of progesterone, shorten life span of corpus luteum and high incidence of silent heat associated with a decrease in conception rate (10-20%) in cattle and sheep in warm climate even good quality and quantity rations were available.

In the present study, changes in the dietary intake from berseem (in winter and spring seasons) or darrhwa (in autumn) only to berseem or darrhwa supplemented with concentrate was associated with a significant improvement in the number of ovulation, recovered embryo/ova, fertilized ova, and the number of grade I transferable embryos. Such improvement may related to the enhanced effect of concentrate supplements in energy, fat and phosphorus levels. Many authors suggested that, negative energy balance or restriction of feed intake reduce response to estrous synchronization, expression of estrus and ovulation rates in cattle (Lucy et al., 1992 and Roberson et al., 1992), sheep (Rhind et al., 1991) and goats (Mani et al., 1992). Furthermore, alteration in dietary lipid could potentially increase the number of follicles available to response to gonadotropins treatment in superovulation regimens (Ryan et al., 1992). Also Little (1975) noted that, ovarian function was improved by providing supplemental phosphorus to grazing cows. Rhind, et al., (1986) reported that, since the effects of nutrition on circulating FSH concentration remains equivocal it also has been suggested that nutrients and nutrient related metabolites that have been implicated in the ovulatory response to nutrition, may operate at the level of the ovary to decrease the amount of FSH needed to support the gonadotropins-dependent follicles

Irrespective to the types of feeding, the present study revealed a positive correlation coefficient were noted between progesterone concentration at the day of embryo collection and ovulation rate, recovered embryo/ova, fertilized ova and Grade I transferable embryo. Also a positive correlation between progesterone prior to hormonal treatment and number of CL and fertilized ova were observed. These results broadly agree with the results of previous work in sheep (Bindon et al., 1971; Eastwood et al., 1976; Oyedipe et al., 1989), Danish Holstein Friesian cows (Greve et al., 1983), Holstein cows (Wubishet et al., 1991), Holstein heifers (Lindsell et al., 1986) and Bohemian x Black-Pied Lowland cows (Peter et al., 1992). They suggested that these parameters are highly related to luteal function at both the initiation of superovulatory treatment and post ovulatory treatments. Further work demonstrated that, deviated concentrations of progesterone or luteinizing hormone or both in the pre-and periovulatory period of superovulated dairy cows was associated with disturbed maturation of ovarian follicles/oocytes, and a lower oocyte recovery rate. They postulated that, asynchrony of oocyte maturation following peak luteinizing hormone may lead to oocyte aging at the time of ovulation and failure of fertilization. Moreover, the improved fertility in cattle has been associated with high circulating concentrations of progesterone during the luteal phase before (Fonseca et al., 1983) and after insemination (Bulman and Lamming, 1978).

Across all treated buffalo-cows in this investigation, a positive correlation was noted between estradiol-17β concentration before treatment and number of follicles of superovulated buffaloes, as well as between estradiol-17β concentration at the day of embryo recovery and no. of follicles and Grade II of transferable embryos. On the other hand, circulating estrogen could considerably influence viability and development of early embryos of buffaloes (Chantaraprateep et al., 1988 and Schallenberger et al., 1990). However, in the present study, no correlation were observed between estradiol before and at the day of embryo recovery, on one hand and ovulation rate on the other hand. These results broadly agree with those of Beg et al., (1996) who did not found correlation between estradiol level and ovulation rate in superovulated buffaloes. Also, Monniaux et al (1983) and Lindsell et al (1986) reported that peak serum estradiol-17β were significantly correlated with observed CL and total ova/embryos but were not correlated with number of fertilized ova or transferable embryos. The presence of large or dominant follicles are associated with changes in endocrine status of the animal (Tamboura et al., 1985; Lussier and Carruthers, 1989; Turzillo and Fortune, 1990 and Guilbault et al 1992) prior to superovulation contribute to a large portion of variability in superovulatory response in cattle. It is well established that, large follicles produce factors that modulate locally and/or systematically growth of other follicles (Findlay et al., 1989 and Monget et al., 1989). In contrast, Guilbault, et al (1992)) found estradiol-17β concentrations measured during the superovulatory treatment were highly and positively correlated with the number of ovulation and the number of growing follicles in superovulated cattle

In the present investigation, a positive correlation were noted between serum glucose before treatment, at the day of embryo collection and the fertilization rate. This result is in accordance with that recorded by Folman, et al. (1973) who reported that, energy deficient diets or negative energy status as reflected by low blood glucose, have adverse effects on fertility. From the results, it was noted that, those buffaloes possess a high concentration of total cholesterol had a high numbers o CL, follicles, recovered embryos and Grade transferable embryos. Also, a positive correlatio was found between total cholesterol level at th day of embryo recovery and fertilized ova. T low level of total cholesterol might indicate th health/energy status of the donors, which in tu would affect the follicular and oocyte maturatio resulting in poor ovulation and embryo yield (B lakrishman et al., 1993). Wehrman et al., (19 and Ryan et al., (1992) recorded that, diet indu hyperlipidemia high (HDL and cholesterol) can affect embryo recovery and viability through its effect on the intrafollicular environment to which the preovulatory oocyte is exposed. It can alter specific aspects of ovarian steriodogenic potential and can increase the population of medium sized follicles theoretically available to respond to gonadotropin treatment in superovulation regimens. Similarly, Kweon et al., (1986) reported that, total cholesterol concentrations of > 90 mg/dl in dairy heifers and > 130 mg/dl in cows were correlated positively with numbers of recoverable embryos from superovulated animals. Moreover, other authors (Balakrishman et al., 1993) found the average number of CL and transferable embryos were significantly lower in donors having < 140 mg/dl than cows having > 140 mg/dl of cholesterol. They also, noticed a significant positive correlation between total cholesterol and the number of CL and transferable embryos and negative correlation between total cholesterol and the No. of unovulated follicles. The same authors found positive energy balance has been associated with high circulating concentrations of progesterone. The large number of unovulated follicles in the low cholesterol suggested that follicular development did occur in response to the superovulatory treatments, but perhaps the function of those follicles were sub-normal, such that the LH surge was not elicited properly and they failed to ovulate (Balakrishman et al., 1993).

In our investigation, concerning the effect of season, comparing group II in winter and spring with group III in summer (the balanced groups) revealed that, the ovulation rates, rate of fertilization and the number of transferable embryos (Grade I) were higher (P<0.05) in winter and spring seasons than summer season (group III). Weathers act as uncontrolled important factor affecting the response of superovulation, ovarian activity and expression of estrus. These parameters were greater in buffaloes in cooler than hot months (Taneja et al., 1995). In Egypt, summer season characterized by increase in the ambient temperature associated with increase in day light and poor nutrition, while in winter, the shortage in the day light is accompanied with decrease in the environmental temperature. Similar results were reported by Tegegne et al. (1993) who found that the number of superovulations which yielded embryos/ova was higher during the wet cool than the dry hot season. Also, the mean number of recovered embryo/ova as well as the number of transferable embryos was being higher during the wet cool than the dry hot season. The differences were attributed to failure of the fimbria to pick up the ova, to degeneration of the ova in the oviduct, or to a possible disturbance in oviduct transport. Ryan et al (1993) concluded that embryonic death increased markedly between day 7 and day 14 of pregnancy during the hot than cool seasons. Moreover, Badinga et al (1992) reported that heat stress appeared to alter the efficiency of follicular selection and dominance and consequence had adverse effect on quality of ovarian follicles.

In conclusion, the results of this study showed that, supplementation of concentrates to the available feeding during winter, spring (Berseem) and autumn seasons (Darrhwa) are necessary to correct the deficiency of some nutrients and can improve ovarian response, embryo recovery, fertilization rate and embryo quality. Seasonal nutrition had a significant effect on the serum hormones (progesterone, estradiol and cortisol) and metabolic status (glucose, cholesterol, albumin, calcium and phosphorous) of superovulated buffalo-cows. Furthermore, the present study suggest that, progesterone and cholesterol concentrations before superovulatory treatment are considered as a good index to expect the ovarian response, embryo recovery and quality of superovulated buffalo-cows.

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