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Seasonal Variations in Superovulatory Response and Embryo Recovery in Buffaloes (Bubalus bubalis)

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ABSTRACT

A total of 11 buffaloes (7 in winter and 4 in summer) were superovulated using follicle stimulating hormone (FSH-P). Flushing was done on day 5/6 using two-way German Catheter and Dulbecco's phosphate buffered saline fortified with bovine serum albumin and antibiotics. Superovulatory response was observed to be better in winter (6.0±0.47 CL) than summer (3.0±0.40 CL). On an average 2.16±0.79 embryos were recovered in winter as against 0.5±0.28 in summer. Based on morphology all of the embryos recovered in winter were of good/excellent quality whereas half of those recovered in summer were good and rest poor.

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Seasonality of reproduction in buffaloes has been reported by a number of reasearchers (Ahmed *et al.,* 1980 Rurki, 1981; Madan and Raina, 1934; Singh *et al., 1988*). However, the information on superovulation and embryo recovery as influenced by the season is lacking. Therefore, the present investigation was undertaken to study the effect of season on superovulatory response and embryo recovery in buffaloes using follicle stimulating hormone (FSH-P; Schering Corporation, USA).

MATERIALS AND METHODS

The investigations were carried out on 11 healthy murrah buffaloes (seven during winter and four during summer seasons) belonging to the dairy herd of Punjab Agricultural University, Ludhiana. Animals with good health and body condition, free from reproductive disorders for the preceding two years, in 2nd to 6th calving but not more than 12 years of age and having crossed the 70th day after the last calving were included in the study. The selected donors were synchronized with 30 mg prostaglandin F_2 alpha (PG, Lutalyse, Upjohn USA) given eleven days apart instramuscularly (I/M). The estrus was detected by parading vasectomized bull at four hourly interval and continuous visual observation for heat symptoms.

All donors were superovulated using 40 mg of follicle stimulating hormone (FSH-P; Schering Corporation, USA) in divided doses twice daily over a period of four days initiating on day 11 (Day O, day of standing estrus) and then induced to estrus by giving two injections of PG 30 mg each I/M administered on 3rd day evening and 4th day morning during the FSH-P injections. The donors were artificially inseminated with fresh semen having more than 80% motile sperms at 8, 20 and 32 hours after the standing estrus with single, double and single dose (1, 2 and 1 ml), respectively. These were palpated on the day of flush (day 5/6) to assess the superovulatory response. The number of corpora lutea (CL) and follicles (> 8 mm) were recorded.

Embryos were recovered non-surgically with the help of two way German Catheter using Dulbecco's phosphate buffered saline (DPBS) containing 0.1% bovine serum albumin

(BSA; fraction V). Embryos were searched under zoom stereomicroscope and transferred to small petri dishes (35 mm) containing holding medium (DPBS + 10% foetal calf serum - FCS).

RESULTS AND DISCUSSIONS

The seasonal conditions under which the experiment was conducted (Table 1) indicate that summer was quite severe and hence stressful.

Estrus Response to Superovulatory Treatment

Prostaglandin-standing heat interval recorded during winter (4.16±2.23 h) was not significantly different from that of summer (29.3±1.75 h) among buffaloes superovulated with FSH. However, the duration of standing-heat was observed to be shorter in summer (20.0±6.27 h)compared to winter season (30.6±9.15 h). By contrast the duration of estrus among buffaloes induced with FSH / PG was observed to be longer during summer (61.5±0.6 h) compared to winter 51.6±0.63 h). Karaivanov (1986) reported a mean prostaglandin-standing heat interval of 44.8±2.31 h and 42.8±1.48 h, respectively in PMSG and FSH induced donor buffaloes in Bulgaria which is comparable with the prostaglandin-standing heat interval recorded in the present study. However, longer standing heat duration in donor buffaloes has been experienced in the present study. The differences may be due to the agroclimatic conditions under which the study was conducted or breed of the donor buffaloes used. Results on duration of estrus in the present study are in accordance with Madan *et al.,* (1988) who have also recorded 60.0±15.4 h in superovulated buffaloes. However, a shorter duration of estrus (24.0±4.0h) has been experienced by Yadav *et al.,* (1988) in buffaloes.

Ovarian Response In Superovulated Donors

Higher total ovarian response (CL+F) was observed during winter (6.16±0.98) compared to summer (4.0±0.40) season (Table 2). It was 54.0 per cent higher in winter than in summer season. These results are at variance to those of Karaivanov (1986) who reported slightly higher ovarian response in buffaloes superovulated with FSH (6.6±2.2) during summer season. This was perhaps due to the fact that the Indian summer is more stressful compared to European summer. The results obtained in the present investigation are in close agreement with Deshpande *et al.,* (1988) who have reported an average total ovarian response (CL+F) of 6.6 follicles during winter months. Significantly ($P < 0.05$) higher number of corpora lutea were detected in buffaloes superovulated in winter (mean 6.0±0.47) than summer (mean 3.0±0.40) in the present study (Table 2). These results are not in agreement with Deshpande *et al.,* (1988) who obtained slightly lower CL development in November-December (4.10) superovulated buffaloes than those in March-April (5.3). Karaivanov (1986) reported the mean number of CL in FSH group of buffaloes as 3.6±1.0 and 5.6±3.78 for spring and summer seasons, respectively.

In cattle also, significantly ($P < 0.05$) less number of CL developed while superovulated during summer compared to other seasons of the year (Almeida, 1987; Gordon*et al.,* 1987). However, Kim *et al.,* (1985) encountered on an average 7.3±1.01, 5.1±1.22, 5.8±1.64 and 4.5±1.39 CL during spring, summer, autumn and winter seasons, respectively.

Embryo Recovery and Quality

The total number of embryos recoveed and transferable embryos per donor buffalo were 2.16±0.79, 2.1 in winter and 0.50±0.28, 0.5 in summer with overall values of 1.5±0.54, 1.4 in the present study (Table 3). The differences observed in the number of embryos recovered and transferable embryos per donor in summer as compared to winter were statistically significant ($P < 0.05$). The results obtained in the present study are not in accordance with Karaivanov (1986) who has reported higher embryos recovery per donor in summer (2.8±1.58) compared to spring (1.1±0.50) from FSH superovulated buffaloes. This could be due to the fact that the European summer is comparable to the favourable season for buffaloes in India. The differences may also be due to inter-lab variation since these results are also not in agreement with Deshpande *et al.,* (1988) who recovered higher number of embryos (24 vs 1, embryos) in trial I (March-April) compared to trial II (November-December). In the present study excellent (30.7%) and good (69.3%) quality embryos were recovered during winter whereas during summer season the quality of embryos recovered, varied from good (50.0%) to poor (50.0%). However, the low embryo recovery rate during summer and winter (16.6% vs 35.0%) indicates the scope of further improvement.

In cattle a tendency of producing more fertilized and transferable embryos in the fall and the spring months has been observed in both **Bos taurus & Bos indicus** (Massey and Oden, 1984). Seasonality in superovulatory response, embryo recovery and quality of embryo have also been suggested previously (Hasler, *et al.,* 1953).

Seasonal changes in the environment mediate their effect on reproduction in a number of ways. Elevated temperature can alter reproductive function in farm animals, which results in extension or shortening of estrus cycle, less intense behavioural estrus as well as reduced fertility (Gangwar *et al.,* 1965). Further more, heat stress has been shown to reduce the basal level and preovulatory surge of LH and has been associated with depressed progesterone level in lactating cows (Madan and Johnson, 1973). The fertility in buffaloes is also significantly influenced by the climatic conditions (Madan and Raina, 1984). It is concluded that the superovulation in buffaloes is poorer during summer season compared to winter under Indian conditions.

Table 1. Climatic Parameters in Different Seasons

Parameters	Seasons	
	Winter	Summer
Maximum Temperature (°C)	21.3 (14.6-26.6)	32.9 (19.0-43.6)
Minimum Temperature (°C)	6.3 (1.2-14.8)	16.3 (4.8-30.4)
Mean Temperature (°C)	14.1 (9.2-19.6)	24.5 (13.8-33.2)
Relative Humidity (%)	73 (55-92)	56 (31-85(
Wind velocity (Kmph)	4.5 (1.7-12.8)	5.2 (1.1-13.6)

Figures in parentheses are the range of the same parameter.

Table 2. Effect of Season on Superovulatory Response (Mean±SE) in Buffaloes Using FSH-P Descending Dose Over 4 Days Period.

Seasons	Donors Responded	Corpora Lutea (CL)	Follicles (F)	CL+F
Winter	6/7	6.0±0.47[a]	0.16±0.04[a]	6.16±0.98[A]
Summer	4/4	3.0±0.40[b]	1.00±0.28[b]	4.00±0.40[B]
Overall	10/11	4.80±0.75	0.50±0.16	5.30±0.50

a.b. Means within column with different superscript differ significantly ($P < 0.05$).

A.B. Means within column with different superscript differ significantly ($P < 0.01$)

Table 3. Effect of Season on Non-Surgical Recovery and Quality of Embryos in Buffaloes Superovulated Using FSH-P in Decreasing Dose Over 4 Days Period

Season	Embryo Recovery			Embryo Quality				Transferable Embryos	
	Total	Mean	Rate	Excellent	Good	Fair	Poor	Mean	Per cent
Winter	13	2.16[a] ± 0.79	13/37 (35.0)	4/13 (30.7)	9/13 (69.3)	0.0	0.0	2.1	100
Summer	2	0.50[b] ± 0.28	2/12 (16.6)	0/2 (0.0)	1/2 (50.0)	0/2 (0.0)	1/2 (50.0)	0.5	50
Overall	15	1.50 ± 0.54	15/49 (30.6)	4/15 (26.7)	10/15 (66.7)	0/15 (0.0)	1/15 (6.6)	1.4	93.3

a.b. Means within column with different superscript differ significantly ($P < 0.01$).

Figures in parentheses indicate percentage.

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