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RESEARCH ARTICLE

Blood plasma hormones in relation to superovulatory response in crossbred cattle

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ABSTRACT

Seven cows in group I and 9 cows in group II were superovulated with 400 mg of folltropin and 18 mg of ovagen, respectively. The blood was collected on day of first estrus (D-0), D-4, D-10, day of super estrus (DOSE) and day of embryo collection (DOEC) from the jugular vein. The blood plasma was analysed for progesterone, estrogen, T3 and T4 by using Elisa kits (Biochem Immunosystems). Superovulation response was higher in the cows superovulated with ovagen than those with folltropin. The number of corpus luteum (CL) and embryos recovered were high in case of cows superovulated with ovagen (8.2 ± 1.4, 3.2 ± 0.5) than with folltropin (7.1 ± 3.4, 1.8 ± 0.6). The progesterone concentration in the blood plasma of highly responded cows (>6 ovulations) was maximum on DOEC whereas it was low in the blood plasma of non responded ones. The animals superovulated with folltropin, which resulted in >6 ovulations and 2 to 7 embryos, the plasma estrogen level was highest on DOSE. In the cows, superovulated with ovagen, resulting in 7-15 ovulations and 3-14 embryos, the blood plasma estrogen level was high on DOEC as compared to low responded ones. T3 and T4 levels were found to be higher in the responded cows than in non-responded ones with both the treatments. These studies indicate that high level of estrogen and low level of progesterone on DOSE can be used as a marker to predict the superovulatory response. It also emphasizes the need of systematic endocrine studies for creation of benchmark(s) and/or biomarkers as diagnostic tools in embryo transfer programme.

Key Words : Hormones, superovulation, embryo, cattle

Embryos collected from superovulated females, vary in developmental stages within the donors, indicating differences in ovulation time, fertilization time and possible developmental problems (Shea, 1981). A reliable method of superovulation is still a significant limitation in embryo transfer in cattle. The embryo yield is highly variable and unpredictable (Greve, 1981). Many investigators have studied the donors in order to control and predict the embryo yield and quality before or at the day of collection using the progesterone /estrogen level as a marker (Tamboura *et al.*, 1985; Yadav *et al.*, 1986; Goto *et al.*, 1988; Aggarwal *et al.*, 1992 and Arosh *et al.*, 2000, 2001). In a number of species estrogen and progesterone are known to affect deleteriously the transport of eggs (Harper and Chang 1971) and alter the properties of the zona pellucida (Dickman, 1969).

Keeping in view, the variable and unpredictable results of superovulation, the present study was planned with the objective to find out a relation between estrogen, progesterone, T3, T4 concentration in blood plasma and number of ovulations and embryo recovery in crossbred cattle, so that a marker can be developed to predict the superovulatory response.

MATERIALS AND METHODS

Sixteen clinically healthy, normally cycling cows, of 4-9 years maintained at the Dairy Farm of Punjab Agricultural University were used for this study. The animals were divided into two groups. Each animal in group I (n=7) and group II (n=9) was given 400 mg of Folltropin and 18 mg of ovagen respectively in four tapering doses, twice daily from day 10 to day 13 of the estrus cycle. On 12th day of cycle, the animals were administered 25 mg of $PGF_2\alpha$. Blood was collected from all the animals from jugular vein on day of first estrus (D-0), D-4, D-10, day of superestrus (DOSE) and day of embryo collection (DOEC). Blood plasma was immediately separated by centrifuging at 6000 rpm for 10 minutes. The plasma was analysed for progesterone, estrogen, T-3 and T4 using Elisa kits (Biochem, immuno systems). The absorbance was read at 450 nm using Elisa reader.

All the animals detected in estrus were inseminated 6,12 and 24 hr after the onset of standing estrus with good quality semen. Embryos were collected non-surgically on

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day -7 with the help of German catheter using Dulbecco's phosphate buffered saline fortified with 0.4 percent bovine serum albumin and antibiotics (Bezylpencillin, Streptomycin) at standard rates.

RESULTS AND DISCUSSION

Superovulatory response : Ninety percent of the cows superovulated with ovagen and 57 percent with that of Folltropin responded to the superovulation treatment. The superovulatory treatment induced 7.1±3.4 (range 0-22) and 8.2±1.4 (range 0-15) ovulations per cow with folltropin and ovagen respectively. In cows superovulated with folltropin on an average, 3.2±0.5 (range 0-7) embryos were recovered. Whereas in the cows superovulated with ovagen 4.3±0.32 (range 0-14) embryos were recovered. A significant correlation (r = 0.85, 0.87) was found between number of ovulations and number of embryos recovered in both the treatment groups.

In the present study, the superovulated cows with higher superovulation response resulted in less number of embryos (Tables 1 and 2). The reason for this may be explained by the hypothesis of Harper and Chang (1971) who have reported that estrogen can accelerate the transport of eggs in the oviduct. It therefore seems likely that in the superovulated cows, the high level of estrogen, which occurs after ovulation could modify the motility of the oviduct and uterus, causing premature transport of eggs into the uterus or expulsion into the vagina. It is also possible that high level of estrogen may bring about premature shedding of the zona pellucida, which would lead to subsequent degeneration of egg (Dickman, 1969)

Hormone profiles in relation to number of ovulations : Hormone profiles in the blood plasma of superovulated animals are described in Tables 1-4.

Progesterone : The concentration of progesterone in the blood plasma of cows superovulated with folltropin, which did not respond was very low (0.25 - 0.5; 0.0 ng/ml) on D-0 and DOEC (Table 1). In these animals, progesterone level was maximum (5.25 - 5.50 ng/ml) on DOSE. Our results correspond with the findings of Maurer and Echterkamp, 1982, who have postulated that the females which failed to ovulate the progesterone concentration in the blood plasma was >1ng/ml only at estrus, but declined to 0.5 ng/ml or less till the DOEC. Datta *et al.* (1992a) have also found average progesterone concentration of 0.46 ng/ml on D-0 in the folltropin treated cows. The progesterone concentration in the blood plasma of cows with more than 6 ovulations (11-22) was maximum (15.5-35 ng/ml) on the DOEC (Table 1). Domeski (1991) reported that in the superovulated cows, the progesterone level decreased rapidly

Table 1. Concentration of Progesterone (P4, ng/ml) and Estrogen (E2, pg/ml) hormones in the cows superovulated with folltropin

Days of Superovulation	Cow Number															
	714		405		373		447		266		642		566			
	P	E	P	E	P	E	P	E	P	E	P	E	P	E		
D_0	5.5	25	1.75	29	2.25	28.1	4.75	35	0.5	36	0.25	11	0.5	25		
D_4	9.25	20	4.75	29	5.75	14	0.1	39	0	83	0.25	0	0.75	59		
D_{10}	2.0	29	5.75	25	9.25	10	4.75	39	3.5	36	4.5	29	3.75	29		
DOSE	0.5	29	0.75	36	0	61	9.25	37	5.5	29	5.25	25	7.75	36		
DOEC	35.0	25	15.5	20	30.5	40	0	37	0	25	0	58	0	61		
No. of CL's	22		11		11		6		0		0		0			
No. of embryos	7		4		2		0		0		0		0			

D_0, Day of First Estrus; DOSE, Day of Super Estrus; DOEC Day of Embryo Collection

Table 2. Concentration of Progesterone (P4, ng/ml) and Estrogen (E2, pg/ml) hormones in the cows superovulated with ovagen

Days of super-ovulation	Cow Number																			
	446		551		568		655		773		707		317		718		700		706	
	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E
D_0	4.25	83	0	105	0.25	50	4.5	120	0.5	36	0	150	0.75	272	0.5	29	0.25	250	6.0	61
D_4	5.50	144	0.1	83	0	36	5.0	100	1.75	36	0.5	133	0.75	29	1.75	122	0	133	0	50
D_{10}	3.75	150	1.25	188	0	50	4.25	122	0.75	61	2.25	144	.5	25	2.5	177	1.0	177	0	88
DOSE	4.25	83	2.0	83	6.75	22	5.75	88	0.75	36	2.0	29	2.0	217	0.25	272	0.75	25	3.5	83
DOEC	5.5	272	4.25	122	20.75	355	5.0	272	29.75	83	12.0	250	7.25	47	3.0	144	3.75	122	0.1	80
No. of CL's	15		15		14		12		10		7		6		2		1		0	
No. of embryos	3		5		13		14		0		4		1		1		2		0	

D_0, Day of First Estrus; DOSE, Day of Super Estrus; DOEC. Day of Embryo Collection

after treatment with $PGF_2\alpha$. They further indicated that the animals which produced good quality embryos had a progesterone level of 0.1 ng/ml at estrus and those produced degenerated embryos had a value of 2 ng/ml. There was a strong correlation (range 0.71-0.89) between progesterone concentration and number of ovulations on D-0, D-4, D-10, DOSE and DOEC in the cows superovulated with folltropin. Similarly, a significant correlation was found between the progesterone concentration and viable embryos on DOSE by Gradela *et al.* 1994, but they found a low and non-significant correlation in the cows, in which blood plasma progesterone concentration was >1.0 ng/ml. On the contrary, the progestrone concentration on day of superoestrus, on day 3 post superoestrus and the day of embryo collection was significantly correlated with number of ovulations, total embryos and transferrable embryos of FSH treated crossbred cows (Arosh *et al.*, 2000).

Arosh *et al.* (2001) also found a positive correlation between number of ovulations, total embryos, transferrable embryos and progesterone concentration on the day of initiation of superovulation, during superovulation and on the day of embryo collection in FSH treated cows.

The cows superovulated with ovagen, which did not respond, the plasma progesterone concentration was minimum (0.1 ng/ml) on DOEC (Table 2). However, the animals with more than six ovulations showed maximum plasma progesterone concentration (5-29.75 ng/ml) on the DOEC. The progesterone concentration in the blood plasma of these animals was very low (0.75-6.75 ng/ml) on DOSE. Datta *et al.* (1992b) have also observed low levels of plasma progesterone concentration on the DOE (0.97 ng/ml) and higher levels on DOEC (9.05 ng/ml) in the folltropin treated cows. The cows resulting in 1-2 ovulations and 1-2 embryos showed the same trend of progesterone levels on DOSE and DOEC as in case of more than 6 ovulations, but the concentration was comparatively low i.e. 0.25-0.75 and 3-3.75 ng/ml (Table 2). Singh *et al.* (1997) opined that the plasma progesterone concentration on D-10, D-12, DOSE and DOEC did not differ significantly in superovulated, normal and repeat breading cows. The rise in progesterone level on D-10 to DOSE in the poor responding cows during the present studies and rise in progesterone levels from D-10 to D-18 in the studies by Booth *et al.* (1975) can be due to gradual lutinisation of follicles. There is also possibility that initial crop of CL might have regained some capacity to synthesize progesterone. There was also a strong correlation (r = 0.71 - 0.89) between progesterone concentration and number of ovulations throughout the treatment in the cows superovulated with ovagen. Similar to

our observations, Moraes IA-de *et al.* (1995) and Takedomi *et al.* (1995) have found a significant correlation between progesterone level, quality and quantity of embryos on the day of initiation of superovulation, estrus and on DOEC. Peter *et al.* (1992) have also found a positive significant correlation (0.70) between the frequency of progesterone pulses, number of ovulations and number of CL and good quality embryos.

Estrogen : The estrogen level in the plasma of cows superovulated with folltropin, which did not respond was very high (58-61 pg/ml) on DOEC (Table 1). The cow # 266 (non responded) was an exception showing decreasing levels of estrogen from the 4th day of superovulation to DOEC. Reverse was the case with cows having developed more than 6 CL, the estrogen level reached a peak (29-61 pg/ml) on DOSE and then suddenly declined (20-40 pg/ml) on DOEC (Table 1). Similarly Datta *et al.* (1992a), have observed that estradiol concentration was at the peak (13.62 pg/ml) on day of commencement of FSH treatment and declined to 3.12 pg/ml on DOEC. Maurer and Echternkamp (1982) have also found that estradiol concentration was >5 pg/ml for 24 hrs after on set of the estrus and declined to <5 pg/ml thereafter. Therefore, our results correspond with the findings of Yang *et al.* (1994), who found that cows with an estradiol 17β level of >30 pg/ml on DOE, produced more CL and transferable embryos in response to superovulatory treatment than cows with lower values.

In cows, superovulated with ovagen resulting in two or ten ovulations and yielding 0 and 1 embryo, the estrogen level in the plasma was at peak (83, 273 pg/ml) on DOSE. Reverse observations were obtained in case of cow # 700 (1CL, 2 embryos) in which the plasma estrogen concentration was 125 pg/ml on DOEC (Table 2). There was an exceptional decline in the plasma estrogen concentration (217 to 47 pg/ml) from the DOSE to DOEC in case of cow # 317. In the cows resulting in 7-15 ovulations and 3-14 embryos, the blood plasma estrogen level was high on DOEC (122-355 pg/ml) as compared to low responding cows (47-144 pg/ml) (Table 2).

In some of the cows, a sudden fall in the concentration of plasma estrogen was noted on D-4 of the cycle (Tables 1, 2). Similar fall was also observed by Seamark *et al.* (1974) on D-6. The fall in estrogen level around D-4 / D-6 may be due to some intrinsic involutionary process in the large follicle, as there is loss of aromatase and desmolase enzyme system around D-6 in follicular cells, which results in the decreased synthesis of estrogen and increased production of progesterone associated with atresia or luteinisation of the follicles (Seamark *et al.*, 1974).

Although a strong correlation (r = 0.58-0.95) was found between the estrogen concentration and number of ovulations in case of ovagen treated cows on D-0, D-4, D-10, DOSE and DOEC, but there was a weak correlation (r = 0.36 - 0.38) in case of folltropin treated cows. Agarwal *et al.* (1992) have also attributed a positive correlation between plasma concentration of progesterone, estradiol, ovulation rate, number of total eggs, fertilized eggs and transferable embryos on day of flushing and day of estrus respectively in cows, superovulated with FSH. A significant correlation (r = 0.83) was found between the plasma estrogen concentration on D-14 and number of viable recovered embryos per cow, and for cows with a plasma progesterone concentration of 1ng/ml on DOSE. Whereas in cows with a plasma progesterone concentration of >1.0 ng/ml, the correlation was low and non-significant (Gradel *et al.*, 1994). According to Arosh *et al.* (2001), there was a positive correlation of number of ovulations, total embryos and transferrable embryos with oestradiol concentration on the day of initiation of superovulation, during superovulation and oestrus in FSH treated crossbred cows.

Triiodothyronine (T-3-) : T3 levels in the blood plasma of cows superovulated with folltropin, which did not respond was maximum (0.74-1.95 ng/ml) on day of first estrus (D-0). Whereas in other two cows (566 and 447) resulting in 0 and 6 ovulations respectively, but nil embryo recovery, the level of T3 was maximum (1.75, 4.85 ng/ml) on D-4 with a progressive decline till DOEC (0.85 - 3.2 ng/ml). In the cows resulting in 11-22 ovulations and 2-7 embryos, T3 level was at peak on DOEC (1.65-1.9 ng/ml) (Table 3).

The cows superovulated with ovagen which did not respond, T3 level in the blood plasma was maximum on D-4 (1.65 ng/ml) and DOSE (1.5 ng/ml) and low on D-10 (0.8 ng/ml) and DOEC (1.0 ng/ml). The cows resulting in 2-15 ovulations and 1-13 embryos also showed variable trend of T3 throughout the superovulatory treatment (Table 4). In case of cow number 551 (15 ovulations, 5 embryos), T3 level was maximum (2.65 ng/ml) on D-4 with a sudden decline (1.90 ng) on D-10 and an increase till DOEC (2.75 ng/ml). Cow number 317 (6 ovulations, 1 embryo) was found to be an exception with very low (0.65-10.9 ng/ml) levels of T3 throughout the superovulation process as compared to the other cows with same superovulatory response. There was a strong correlation (r=0.53-0.75) and a weak correlation (0.41-0.50) between T3 levels and number of ovulations in cows superovulated with Folltropin and ovagen, respectively (Table 4).

Thyroxin (T4) : Animals superovulated with folltropin resulting in 11 ovulations and 2-4 embryos showed a gradual increase in T4 levels till DOSE and then a sudden decline on DOEC. One

Table 3. Concentration of T3 (ng/ml) and T4 (mg/dl) hormones in the cows superovulated with folltropin

Days of super-ovulation	Cow Number													
	714		405		373		447		266		642		566	
	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4
D_0	0.95	9.00	0.80	8.85	1.75	10.15	4.85	9.7	0.95	8.85	0.74	10.5	1.40	8.85
D_4	0.75	8.30	0.85	9.00	1.76	7.85	4.85	14.4	0.55	9.70	0.60	9.70	1.75	9.00
D_{10}	0.65	14.62	1.25	14.00	1.65	7.85	4.0	7.5	0.30	8.00	0.60	7.85	1.50	14.00
DOSE	0.75	7.50	1.65	8.85	0.80	10.50	3.40	11.0	1.40	9.00	0.95	8.30	0.95	8.85
DOEC	1.90	8.85	1.65	5.00	1.76	6.80	3.20	9.7	0.75	8.65	0.80	9.70	0.85	5.00

D_0, Day of First Estrus; DOSE, Day of Super Estrus; Day of Embryo collection

Table 4. Concentration of T3 (ng/ml) and T4 (mg/dl) hormones in the cows superovulated with ovagen

Days of super-ovulation	Cow Number																	
	446		551		568		655		773		707		317		718		700		706	
	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4	T3	T4
D_0	0.95	6.5	1.65	7.0	1.6	7.15	0.95	5.50	0.75	6.8	2.05	6.5	0.65	11.5	1.20	7.15	1.25	6.30	1.25	7.85
D_4	1.50	7.5	2.65	7.5	0.95	6.30	1.00	5.20	1.55	7.77	2.20	7.5	0.95	8.85	1.15	10.5	0.80	9.70	1.65	7.00
D_{10}	1.15	7.2	1.90	7.5	0.75	7.15	1.05	5.75	1.10	5.87	2.30	7.2	0.65	11.15	2.20	10.0	1.90	8.77	0.80	7.85
DOSE	1.75	7.0	2.20	5.3	1.55	8.30	1.50	6.85	1.90	8.27	1.40	7.0	0.75	11.5	1.50	9.0	2.05	7.50	1.50	8.27
DOEC	2.20	6.8	2.75	6.5	1.40	8.00	2.05	2.85	1.00	9.70	1.10	6.8	0.95	11.0	0.95	7.85	1.25	3.5	1.00	8.00

D_0, Day of First Estrus, DOSE, Day of Super Estrus, DOEC. Day of Embryo Collection

of the cows resulting in very high superovulatory response (22 ovulations, 7 embryos, T4 levels increased upto D-10 and then showed a sharp decline till DOEC (14.62 - 8.85 mg/dl). The cows, which did not respond to the superovulatory treatment showed a great variation in plasma T4 levels throughout the superovulatory treatment (Table 3).

The cows superovulated with ovagen, which did not respond showed a gradual increase in T4 levels till DOSE (7.85-27 mg/dl) then a little decline on DOEC (8.0 mg/dl). A gradual decline in plasma T4 levels was found from D4 to DOEC in cows with 1-2 ovulations and 1-2 embryos. A similar trend was found in another cow resulting in 15 ovulations and 3 embryos (Table 4). Cow # 551 (15 ovulations, 5 embryos) showed a sharp decline from D-10 (7.5 mg/dl) to DOSE (5.3 mg/dl) and then an increase on DOEC (6.5 mg/dl). Three cows (# 317, 568 & 707) although had a different superovulatory response, but T4 level in their blood plasma was similar (Table 4). A strong correlation (r = 0.51 - 0.80) was found between number of ovulations and T4 levels in the cows superovulated with ovagen. On the other hand a week correlation (r = 0.12-0.13) was observed throughout the treatment in the cows superovulated with folltropin.

The results obtained during the present studies on T3, T4 levels in blood plasma of superovulating cows are quite variable. The variation in the concentration of T3 and T4 may be due to individual response of the animals. High level of T3 and T4 in the blood plasma of responding cows than that of non responding ones may seem to reflect some metabolic changes due to enhanced activity of ovaries.

It can be concluded from the present studies that high level of estrogen and low level of progesterone on DOSE can predict the superovulatory response in the cows. Low level of progesterone at the time of estrus may be explained as the preovulatory surge occurring during estrus (Srenam *et al.*, 1978), the progesterone that is measured could come from preovulatory follicles before luteinisation and ovulation. Saumande *et al.* (1985) have also suggested that it is more important to know the number of embryos to be expected from ovulation rate and for this reason a strong correlation between progesterone concentration and number of collected embryos is of interest. Tamboura *et al.* (1985) have also opined that the percentage of viable embryos can be predicted from progesterone pattern during the superovulatory treatment. Therefore, our observations and the opinion of Saumande *et al.* (1985); Tamboura *et al.* (1985) emphasize the importance of endocrine studies as diagnostic tools for predicting superovulatory response and quality embryo production in embryo transfer programme.

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