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PLASMA ESTROGEN AND PROGESTERONE CONCENTRATIONS IN RELATION TO EMBRYO YIELD IN SUPEROVULATED BUFFALOES

By

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INTRODUCTION

Enormous variability of ovarian sponss following superovula - on constitues the primary factor miting the sucess of embryo ansfer technique in farm animals Herrier et al., 1990) . It is of reat interest in embryo transfer rograms, prior to induction of su- erovulation, to predict the donors sponding with poor ovulation tes. This, perhaps, would contrib- te to substantial savings of finnan- ial and time resources. In cattle, everal studies (Booth and New- omb, 1975; Saumande et al., 985; Tamboura et al, 1985; and aumande and Chupin, 1986) ave shown that progesterone and stradiol levels in the plasma are elated to superovulation response. Moreover, ovulation rate and em- bryo production can be predicted by the plasma levels of these hor- mones (Saumande, 1980; Greve et al.1983 and Goto et al., 1987) In buffaloes similar information are scanty. The present work, therefore, was conducted to study the progesterone and estradiol profiles in relation to the ovarion response in superovulated buffaloes. In addition, the relationships between plasma levels of these hormones during the peri-ovulatory time and each of the ovulation rate and number of recovered embryos were considered.

MATERIALS AND METHODS

Thirteen adult, non-pregnant and non-lactating buffalo cows of 6 to 11 years old were included in this study. The animals were raised under similar conditions in two locations; Animal Reproduction Research Institute, Giza province and El-Khanka governmental farm, Kalubia province. All animals were synchronized using one or two doses-11 days apart-of 25 mg prostaglandin $F_2\alpha$ (Lutalyse, Upjon Co.). At the mid-luteal stage they were treated with a total dose of 34 mg FSH-P (Scherng corporation, USA) for four consecutive days (7, 7, 5, 5, 3, 3, 2 and 2 mg). With the

5th and 6th doses of FSH two doses of 25 mg FGF_2 α were administrated to induce luteolysis, then at estrus they were naturally mated with a fertile bull. Embryo recovery was carried out non-surgically on the 6th day of the cycle using the method described by **Newcomb et al. (1978)**. The numbers of follicles and corpora lutea were counted by rectal palpation as the mean of two separate estimates.

Blood samples (about 20 ml) were collected in heparinized tubes by jugular venipuncture early in the morning. Sampling period started from te first day of FSH treatment (day-4) and continued daily up to the day of embryo recovery (day 6 post-estrus). Plasma samples were stored at -20°C pending hormonal analysis.

Pleasma estradiol-17B was measured according to the method adopted by **Landgren et al. (1982)** whereas plasma progesterone was analysed using the method employed by **Sheehan et al. (1982)**. Both hormones were measured using Coat-A-Count kits of Diagnostic Products Corporation, USA. Gamma counter (Berthold) was used for counting and the produced number was converted by the way of calibration curve for measuring both hormones in unknown samples. The sensitivity of the assay, defined as the smallest concentration significantly ($P < 0.05$) distinguishable from zero was 0.1 ng/ml and 3 Pg/ml plasma for progesterone and estrogen hormones, respectively. Intra-and inter assay coefficients of variation were 7.9 and 8.5% for progesterone and 12.3 and 16.8% for estrogen hormones, respectively.

Differences in mean values were tested by student's t-test. Correlation coefficients were calculated between the levels of hormones and studied parameters according to **Snedecor and Cochran (1976)**.

According to **Karaivanov (1986)**, non-responding buffaloes are those showing two or less corpora lutea as a result of the superovulatroy treatment.

RESULTS

As depicted in Table 1, four buffalo cows (30.77%) did not respond to the superovulatory treatment (showed two or less CL). The mean numbers of corpora lutea and unovulated follicles in these animals (G I) were 1.25 ± 0.42 and 0.75 ± 0.42, respectively. No embryo recovery was carried out for the buffaloes of this gorup. On the other hand, nine buffaloes (69.23%) responded to FSH treatment by experiencing more than two corpora lutea. The mean numbers of corpora lutea and unovulated follicles in these donors (G II) were 5.89 ± 0.40 (range 4-8 CL) and 0.89 ± 0.25 (range 0-2follicles), re

Table (1) : Plasma progesterone and estrogen concentrations in relation to the ovarian response and embryo production (Mean ± SEM) in buffalo donors.

Group	No. of animals %	First day treatment		Day of estrus		Day of recovery		Overian response		No. of recovered embryos
		P_4	E_2	P_4	E_2	P_4	E_2	CL	F	
G I	4(30.77)	0.90^a ± 0.20	4.58^a ± 0.67	0.18^a ± 0.02	6.35^a ± 0.71	1.70^a ± 0.33	4.48^a ± 0.39	1.25^a ± 0.42	0.75^a ± 0.42	No recovery
G II	9(69.23)	3.38^b ± 0.30	4.82^b ± 0.56	0.14^b ± 0.03	16.28^b ± 1.23	5.46^b ± 0.38	4.74^b ± 0.31	5.89^b ± 0.40	0.89^b ± 0.25	2.56 ± 0.42

P4 = Progesterone ng/ml
E2 = Estradiol-17ß pg/ml
Cl = Corpora lutea
F = Unovulated follicles >10mm.
Values with different superscript in the same column differ significantly (P<0.01).

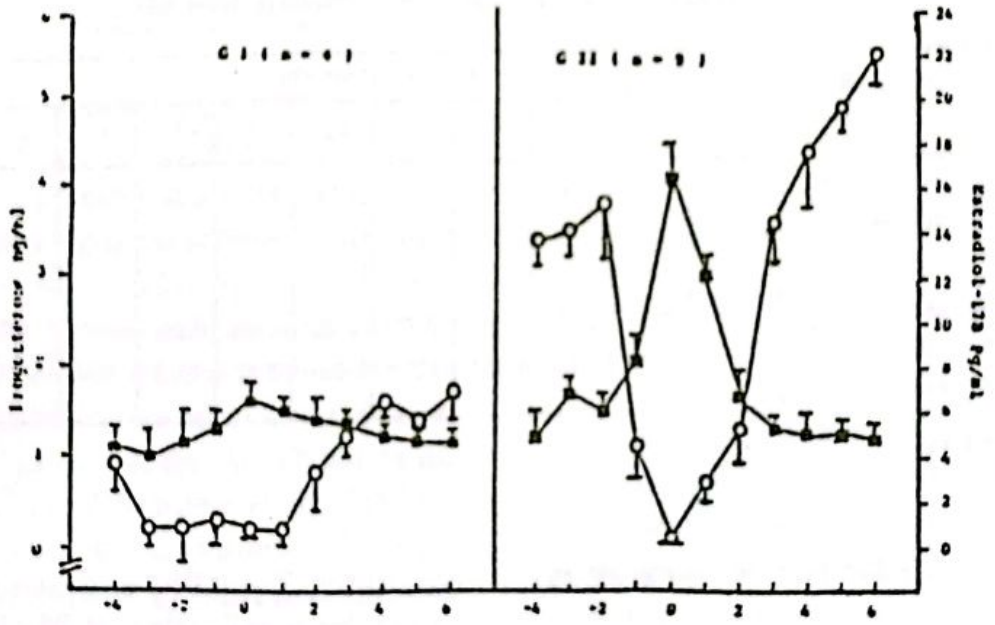

Fig.(1)

spectively. Difference in the number of corpora lutea between the two groups was highly significant ($P < 0.01$) whereas no statistical difference was noted between the two gorups in the number of unovulated follicles (Table 1). Twenty three embryos were collected from the donors of G II (2.56 ± 0.42) with a range from 0 to 4 embryos. They included 19 morula and four hatched blastocysts. All embryos were classified as morphologically normal and ranged from fair to ex-

cellent in quality.

Plasma progesterone concentration at initiation of superovulation (Table 1) was higher ($P < 0.01$) in G II than G I (3.38 ± 0.30 vs 0.90 ± 0.20 ng/ml). This difference continued during all treatment days (days- 4 to -1). On days 0, 1 and 2 post-estrus no differences were noted, but by day 3 onwards significant differences were detected again (Fig. 1). Meanwhile, Plasma estrogen concentration differed significantly ($P < 0.01$) between the two groups at the day of estrus as well as one day before and after estrus.

Concerning the superovulated buffalo donors (G II), no relationships were found between plasma progesterone or estrogen concentrations at the first day of treatment and the day of recovery, and the number of palpated corpora lutea (Table 2). However, a significant correlation (-0.72, $P < 0.05$) wa obtained between the number c copora lutea and progesterone con centration on the day of estrus Also, significant ($P < 0.05$) corre lations were recorded on day-1 an day 0 between estrogen concentra tion and the number of corpora lu tea. On the other hand, significan correlations existed between th number of recovered embryos an progesterone concentration at initi ation of superovulation (0.82; P < 0.01), at estrus (- 0.77; $P < 0.05$) one day before embryo recover (0.68; $P < 0.05$) and at the day of embryo collection (0.75; $P < 0.05$) Estradiol concentration was alsc correlated with the same criterion (Table2) at the day of estrus (0.71 $P < 0.05$), one day before (0.68; I < 0.05) and on the day of embryc recovery (- 0.73; $P < 0.05$).

The pattern of progesterone and estradiol concentrations of buffa-

Table (2) : Coefficient of correlation between plasma levels of progesterone (P_4) and estradiol-17ß (E_2) and each of the number of corpora lutea and recovered embryos in superovulated buffaloes (G II , n = 9).

		Days of treatment										
		-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6
1) Number of corpora lutea	P_4	0.20	-0.17	-0.39	-0.52	-0.72*	0.08	0.11	0.18	0.13	0.24	0.30
	E_2	0.04	0.12	0.33	0.67*	0.69*	-0.22	-0.19	-0.30	-0.29	-0.32	-0.38
2) Number of recovered embryos	P_4	0.82**	0.33	0.12	-0.39*	-0.77*	-0.21	0.29	0.34	0.40	0.68*	0.75*
	E_2	0.15	0.25	0.44	0.68*	0.71*	0.34	-0.12	-0.25	-0.28	-0.44	-0.73*

* $P < 0.05$
** $P < 0.01$

loes that did not respond (G I) and responded (G II) to superovulation is shown in Fig. 1. In G I there were no significant changes in the estrogen and progesterone profiles from day of FSH treatment (day-4) to day of embryo recovery. In G II progesterone declined after $PGF_2\alpha$ treatment to reach a basal level on the day of estrus. It then showed a dramatic increase in the post-multiple ovulatory phase (day 1 to 7). Estradiol concentration showed a marked increase after $PGF_2\alpha$ administration (day -2) and then declined to basal values from day 1 onwards.

DISCUSSION

Non-responding buffalo cows to exogenous gonadotrophin treatment represent a major problem in developing the embryo transfer technique in this species. In the present investigation, they constitute 30.77% of the treated buffaloes. Similar results were reported in the same species by **Drost et al. (1988) and Madan et al. (1988).** However, higher values (60-88%) were recorded by **Sharifuddin and Jainudeen (1984) and Karaivanov (1986).** The overall mean of the palpated corpora lutea (5.89) in superovulated buffaloes (G II) was fairly close to 3.5 - 5.15 CL given by **Drost et al. (1988), Misra et al. (1990) and Mohamed (1991).** Recently, a higher ovulation rate (7.5 CL) was estimated through the ovarian dissection of buffalo donors **(Karaivanov et al., 1990).**

The highly significant difference between the two bufalo groups in progesterone concentrations at initiation of superovulation indicates that the ovarian function especially the function of corpus luteum on the first treatment day has a key role in successful superovulation in buffaloes. Similaly, **Tuyen et al. (1990)** found that buffalo cows with low progesterone level at the time of superovulation showed poor response. In cattle, **Goto et al. (1987)** observed significant differences in the ovulation rate, number of recovered embryos as well as number of normal embryos between cows with progesterone levels less and more than 3 ng/ml at first day of superovulation. In this aspect, **Callesen et al. (1988)** reported that higher levels of progesterone at start of superovulation tended to supress the basal LH discharge from initial injection of gonadotrophin to injection of prostaglandin; this allowed for greater storage of LH and subsequently produced a broader LH surge with a higher peak level. Such an LH discharge pattern has been found to be favourable in terms of ovulation rate and embryo quality **(Jensen et al., 1982 and Donaldson, 1985).** The significant difference in progesterone levels at the day of recovery between the two groups is probably related to the difference in the number of

corpora lutea. This concept is supported by the highly significant difference in the number of corpora lutea palpated in both groups on the day of recovery. At the same time, the great variation in estradiol concentration at day of estrus between the two groups is presumably due to the variation in number of mature follicles present at that time. **Hendricks and Lamond (1972)** reported that plasma estrogen levels were greater in cows with multiple ovulations than in cows with single ovulation.

In agreement with the findings of **Jensen et al. (1982), Greve et al. (1983), Saumande et al. (1985) and Goto et al. (1987)** a highly significant relationship was found between progesterone level of the initiation of superovulation and the number of recovered embryos. This indicates that measurement of progesterone concentration on first day of superovulation can be used as a reliable method in predicting the embryo yield in buffalo species. On the other hand, **Walton and Stubbings (1986)** found that there was no relationship between the concentration of progesterone at start of superovulation and the number of recovered embryo. Moreover, a negative correlation between the increase in progesterone concentration two days after the beginning of treatment and the percentage of transferable embryos was observed by **Tamboura et al. (1985)**. In this study, the finding that no relationshp existed between progesterone level at the start of superovulation and the subsequent ovulation rate was similar to that reported by **Sreenan and Gosling (1977), Saumonde (1980), Greve et al. (1983) and Walton and Stubbings (1986)**. The presence of luteinized follicles in superovulated animals may-in part-has a role in this relationship. **Monniaux et al. (1983)** reported that in superovulated animals, unless by histological examination, it is difficult to distinguish the corpora lutea and luteinized follicles no matter which technique is used; direct observation, endoscopy or rectal palpation.

Speculative analysis of the hormonal profiles in the two groups of buffalo cows revealed that in G II (buffaloes responding to superovulation) progesterone level showed slight elevation following to FSH-treatment, then decreased within two days after $PGF_2\alpha$ treatment to reach a basal value at day of estrus. Thenceafter, it increased rapidly after ovulation to reach a peak value on day of recovery. Similarly, **Saumande et al. (1985)** reported that as the number of corpora lutea increased, the progesterone level rose faster and higher for any given day after ovulation. In agreement with **Tuyen et al. (1990)** in buffaloes, estradiol concentration showed a gradual increase after FSH - treatment to reach its maximum level at day of estrus. The

arked relationship between es-adiol level on day-1 and day of strus and the number of recovered mbryos is a clear indication of a gh number of periovulatory folli-les. **Saumande and Chupin (1986)** have documented that the aximum concentration of estradi-l was correlated with the number f 15 mm follicles recorded at es-rus. Hormonal profiles of G I non-responding buffaloes) did not how any significant changes in re-and post-ovulatory phase. Sim-lar findings were reported by **Saumande et al. (1985), Goto et al. (1988) and Mehmood et al. (1991).** The latter authors added hat this hormonal profile is a clear ndication of ovaries which are on-responsive to exogenous gona-otrophin.

SUMMARY

The relationships between plasma es-radiol-17 β and progesterone concentra-ions and each of the ovarian response and mbryo yield were investigated in thirteen dult buffaloes. Superovulation was in-uced in thes animals using FSH in com-ination with prostaglandin F2 α. Blood amples were collected daily for hormonal nalysis from the day of treatment (day-4) o the day of embryo recovery (day 6 ost-estrus).

The results revealed that 30.77% (G I) f the treated buffaloes did not respond to he superovulatory treatment (developed wo or less copora lutea) whereas 69.23% (G II) responded to FSH-treatment by ex-periencing more than two corpora lutea. Progesterone (P_4) concentration at the ini-iation of superovulation was higher ($P < 0.01$) in G II than in G I. This difference continued during all treatment days (days -4 to -1) and reappeared at day three post-estrus onwards. Estradiol-17B (E_2) level differed significantly ($P < 0.01$) between the two groups on the day of estrus as well as one day before and after estrus. In G II, significant correlations existed between the number of recovered embryos and P_4 concentration at the initiation of superovulation, at estrus and on the day of embryo recovery. This revealed that P_4 level at the start of superovulation can be used as a reliable method to predict the embryo yield in buffalo species. E_2 concentration was correlated with the number of recovered embryos on the day of estrus and on the day of embryo collection. On the other hand, no correlations were found between P_4 or E_2 levels and the ovulation rate on the first day of treatment or on the day of embryo recovery.

Hormonal profiles in buffaloes of G II showed marked changes in pre-and post-ovulation phase. However, in animals of G I no significant changes in hormone concentration were noted and this was considered a clear indication of the ovaries that are non-responsive to exogenous gonadotrophin.

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شركة المهن الطبية للمنتجات البيطرية وإضافات الأعلاف

إحدى شركات المهن الطبية للإستثمار المنبثقة من إتحاد المهن الطبية .

وهى شركة مساهمة مصرية تأسست سنة ١٩٨٣ برأس مال عربى إجنبى بتكاليف إستثمارية قدرها ٨ مليون جنيه مصرى . ورأس مال مملوك قدره ٤ مليون جنيه مصرى .

ويتضمن نشاط الشركة : -

١) تصنيع مركزات الاعلاف " دواجن - ماشية "

٢) تصنيع إضافات الاعلاف المحتوية على الفيتامينات والاحماض الامينية و الاملاح المعدنية وكذلك المضادات الحيوية ومضادات الطفيليات .

٣) تصنيع وتعبئة ومعايرة اللقاحات " دواجن - ماشية " .

٤) تصنيع وخلط وتعبئة المطهرات ومضادات الطفيليات الداخلية والخارجية .

٥) تصنيع مختلف الادوية البيطرية .

٦) تصنيع بدائل الالبان .

٧) القيام بتسويق هذه المنتجات فى الداخل وفى الخارج .

وتقع مصانع الشركة بمنطقة أبو سلطان الإسماعلية وهى على أحدث تكنلوجيا عالمية لإنتاج منتجات على أعلى مستوى من الجودة تضارع مثيلاتها الاجنبية إن لم تتفوق عليها .

الإدارة : عمارة ١٦ المنطقة الخامسة - عمارات شركة مصر للتعمير

المصانع : أبو سلطان - الإسماعيلية ت : ٢٦٩٧٠٦٨

فاكس : ٢٦٩٧٠٦٧