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HORMONAL PROFILE ASSOCIATED WITH ESTABLISHMENT OF PREGNANCY IN FRIESIAN COWS IN RELATION TO SUMMER CONDITION IN EGYPT

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SUMMARY

The present study was carried out on 16 multiparous Friesian cows inseminated during summer season (June and August) to compare between the hormonal profile of pregnant and non-pregnant cows within the period of pregnancy establishment (maternal recognition). Estrus was checked three times daily, and a cow that was recognized to be on heat was inseminated with frozen semen 8-16 hrs from the beginning of heat. Blood samples were collected daily from the day of insemination up to day 21 post-insemination to determine the concentrations of progesterone, while $PGF_{2\alpha}$ and PGE_2 concentrations were determined throughout the period from day 12 to day 20 (period of maternal recognition in pregnant cows). Pregnancy diagnosis took place 45 days post-insemination using rectal palpation. According to the results of pregnancy diagnosis, animals were allocated into two groups (n = 8 each), pregnant (G_1) and non-pregnant (G_2).

Results indicated that pregnant cows had higher levels of progesterone and PGE_2 than cows of G_2, while $PGF_{2\alpha}$ levels was lower than in non-pregnant ones. During the period of maternal recognition (day 16 and 17) $PGF_{2\alpha}$ / PGE_2 ratio was 1:4 and 1:7 in G1 and G2, respectively.

Keywords: Friesian, maternal recognition, progesterone, $PGF_{2\alpha}$, PGE_2

INTRODUCTION

Season of year has a significant influence on embryonic mortality (Hansen *et al.,* 2001) and reproductive failure (Hansen and Arechiga,1999). Friesian cattle were introduced to Egypt to bridge the gap of milk demand and supply. Previous studies indicated that their reproductive performance is lower during summer of Egypt compared to winter (El-Keraby and Aboul-Ela, 1982) which could be most probably due to the effect of heat stress (Hansen *et al.,* 2001 and Al-Katanani *et al.,* 2002)

Embryonic mortality before day 18 of pregnancy is one of the major reasons causing reproductive dysfunction in cattle (Dunne *et al.,* 2000). One of the reasons of this phenomenon is the failure in luteotrophic mechanism(s), which is necessary to keep high concentration of progesterone (P_4) during pregnancy (Mann and Lamming, 2001; Spencer and Bazer, 2002 and Inskeep, 2004). Luteotrophic mechanism is a complicated process, in which pituitary gland, embryo, uterine endometruim and ovaries are share in.

Many studies were conducted to describe the hormonal balance during pregnancy with particular emphasis on the time of maternal recognition (days 12-18 post insemination) in to understand the luteotrophic mechanism in cattle (Schams and Berisha, 2004). Oyedipe *et al.* (1986) and Peters (1996) found that P_4 was higher in pregnant than in non-pregnant cows due to maintenance of corpus luteum (CL). Parent *et al*. (2002) and Inskeep (2004) reported that PGE_2 concentration is greater in pregnant than in non-pregnant animals during day 17 after insemination. On the contrary, $PGF_{2\alpha}$ was less in pregnant than in non-pregnant one (Asselin and Fortier, 2000 and Parent *et al.,* 2002).

Up to the knowledge of the authors, no data are available to describe the hormonal balance during the maternal recognition period of Friesian cattle under Egyptian summer environment, which was the aim of the present study.

MATERIALS AND METHODS

Animals and Management

The present study was carried out at EL-Karaada Animal Production Research Station, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Egypt, in the Nile delta. Purebred multiparous Friesian cows (between the 2^{nd} and 4^{th} parities and 4-6 years of age) inseminated during June and August were used in this study. Estrus was observed three times daily and cows in heat were inseminated with frozen semen 8-16 hrs after the beginning of heat.

Cows were housed in a semi-shaded yard and fed on dry feed composed of concentrate mixture plus rice straw to cover their requirement calculated according to NRC (1988) allowances. Clean water for drinking was available all the time. Dams were allowed to nurse their calves four days pot-partum before being machine milked twice daily

Pregnancy was checked 45 days post-insemination applying rectal palpation. After pregnancy diagnosis eight pregnant cows (G1) and eight non-pregnant ones (G2) were allocated as two experimental groups.

Blood Sampling and Hormonal Assay

Daily blood samples were collected from day 1 to day 21 post-insemination before being collected regularly at 3 - 4 day interval till day 45 post-insemination. In the case of estrus return during the 45 days post insemination, sampling collection was discontinued and the cow was considered non-pregnant. Blood samples were collected before drinking in the morning (07:30 hr) from the jugular vein into heparinized tubes. Progesterone was measured throughout the period from day 1 to day 20 days post-insemination, and $PGF_{2\alpha}$ and PGE_2 concentrations were determined throughout the period from day 12 to day 20 days post-insemination (maternal recognition period; Peters, 1996). After collection, samples were centrifuged at 3000 rpm for 15 minutes for plasma separation before being stored at -20 C till the time of assay.

Plasma progesterone (P_4) concentration was carried out using ready coated radioimmunoassay kits (Immunotech, France). The sensitivity of the assay was reported to be 0.03 ng/ml. Intra- and inter-assay coefficients of variation were 4.3 and 8.2 %, respectively. The standard curve ranged between 0.1 and 50 ng/ml. while the cross reaction of the antibody was 8.8 % .

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) concentrations were measured using Correlate-EIA assay (A competitive immunoassay kit, Assay Designs Inc., USA). The values of $PGF_{2\alpha}$ and PGE_2 standard curves ranged from 3.0 pg/ml to 50 pg/ml. Sensitivity value of the assay was reported to be 6.8 pg/ml. Cross reaction was 3.6 and 0.8 % against $PGF_{2\alpha}$ and PGE_2 , respectively and <0.1 % for the other metabolites of prostaglandin. The intra- and inter- assay variation coefficients were 8.2 and 6.1, respectively.

Statistical Analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS linear program (1996). Differences between two between means of the experimental groups were tested for significance using Duncan's New Multiple Range Test (Duncan, 1955). The area under $PGF_{2\alpha}$ and PGE_2 curve throughout the period from 12 to 20 days post-insemination was calculated by Autocad program (2000). These data were analyzed according to the following model :

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

Y_{ij} = any j^{th} observation in i^{th} group,
μ = overall mean,
G_i = effect of i^{th} group i = 1-2,
e_{ij} = random error.

RESULTS

1-Progesterone Concentration

Within the 21 days post- insemination, progesterone (P_4) increased to reach the level of ≥ 1.0 ng/ml between days 4 and 6 before increasing exponentially to reach its maximum concentration at day 11 in non-pregnant cows (G_2). P_4 continued to increase up to the end of the experimental period (> 6.0 ng/ml around day 21) in pregnant ones (G_1) (Figure 1).

According to the trend of increase of P_4 in G2, the pattern could be divided into three phases; ascending (day 0- day 10); plateau (day 10 – day 17) and descending (day 17 – day 21). These phases were not corresponded in G1, where only two phases were detected; the ascending one (day 0 – day 14) and plateau (day 14 – day 21).

P_4 concentration in G1 at day five was about three times higher than in G_2 (2.8 ng/ml *vs.* 0.8 ng/ml). Area under P_4 curve in G_1 was higher ($P<0.05$) than in G2 during the period from day 3 to day 8 and from day 11 to day 21,while it was insignificant during rest days.

2- Prostaglandins Concentration

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations were lower in G_1 than in G_2 during the period from day 12 to day 20 post-insemination. The maximum variation in concentration $PGF_{2\alpha}$in the two studied groups was observed at day 17 (Figure 2 and Table 1) post-insemination (time of starting descending phase of P_4 in non-pregnant cows).

In contrast, prostaglandin E_2 (PGE_2) concentration was higher in G_1 than in G_2 between day 12 and day 20 post-insemination. The maximum difference of PGE_2 concentration between G_1 and G_2 was observed at day 16 post-insemination (Figure 3 and Table 1).

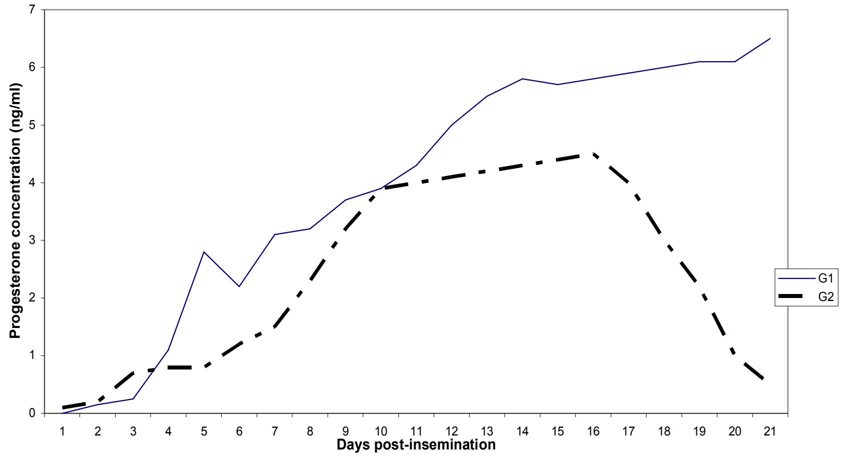

Figure 1: Progesterone concentration (ng/ml) in pregnant (G1) and non-pregnant (G2) cows during the first 21 days post-insemination

PGE_2 / $PGF_{2\alpha}$ ratio during the period from day 12 to day 20 post-insemination was higher in G_1 than in G_2 (Figure 4). The interesting point to be noted is that PGE_2/ $PGF_{2\alpha}$ ratio at day 17 post-insemination was 2.8 ± 0.1 in G_1 *vs.* 0.4± 0.1 in G_2. In G_1 PGE_2/ $PGF_{2\alpha}$ ratio at day 16 and 17 post-insemination was 4.2 and 7 folds, respectively, in comparison with cows of G_2. Another interesting finding is that the area under PGE_2 curve throughout the period from 12 to 20 days post-insemination was insignificantly larger in G_1 than in G_2, (7.89 *vs.* 5.87 arbitrary units of cm^2, respectively), while the area under $PGF_{2\alpha}$ curve had a contrasting trend (5.06 *vs.* 8.07 arbitrary units of cm^2 in G_1 and G_2, respectively). This may indicate the luteolytic activity in G_2 than G_1.

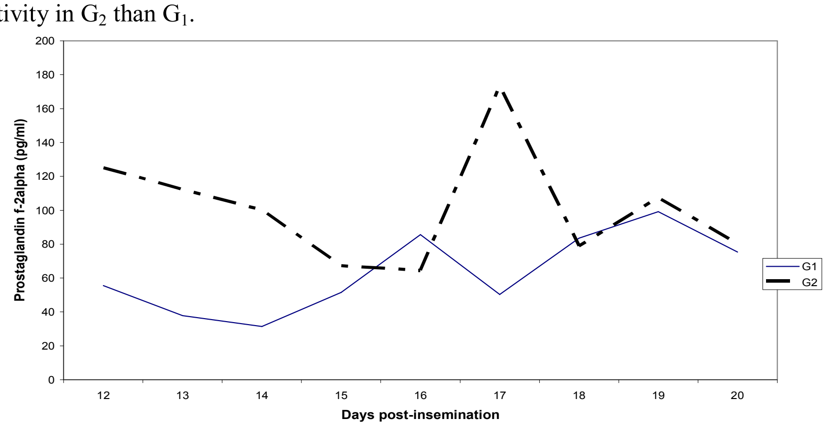

Figure 2: Prostaglandin F-2 alpha concentration (pg/ml) in pregnant (G1) and non-pregnant (G2) cows during the period from day 12 to day 20 post-insemination

Table 1. Means (± SE) of $PGF_{2\alpha}$ and PGE_2 concentrations (pg/ml) in pregnant (G1) and non-pregnant (G2) Friesian cows from day 12 to day17 post-insemination

Days	$PGF_{2\alpha}$		PGE_2	
	G_1	G_2	G_1	G_2
12	55.6±26.1^{a}	125.1±16.3^{ab}	85.1±22.4^{a}	136.1±43.5^{ab}
13	37.8±12.0^{a}	112.3±48.3^{ab}	123.4±43.4^{a}	94.0±41.4^{ab}
14	31.4±10.4^{a}	100.2±45.3^{ab}	105.3±41.9^{a}	108.4±40.7^{ab}
15	51.5±19.9^{a}	67.3±24.2^{ab}	82.8±24.8^{a}	69.1±33.1^{ab}
16	85.6±35.2^{ab}	64.6±16.3^{ab}	156. ±26.0^{a}	40.4±15.3^{b}
17	50.3±28.4^{a}	173.3±36.3^{b}	123.3±13.6^{a}	70.2±40.2^{ab}

a,b in the same row means with different superscripts are significantly different ($P < 0.05$)

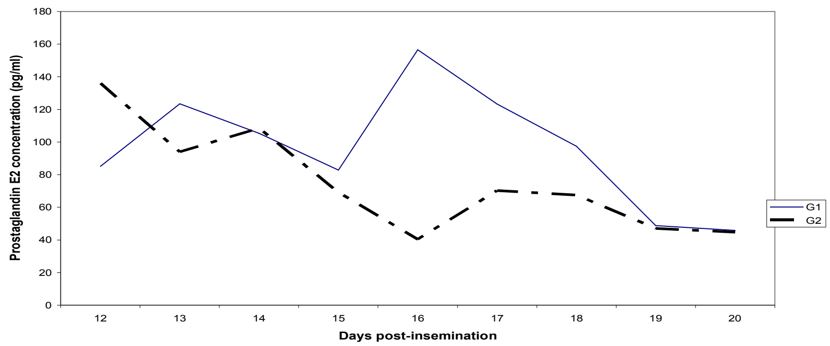

Figure 3: Prostaglandin E2 concentration (pg/ml) in pregnant (G1) and non-pregnant (G2) cows during the period from day 12 to day 20 post-insemination

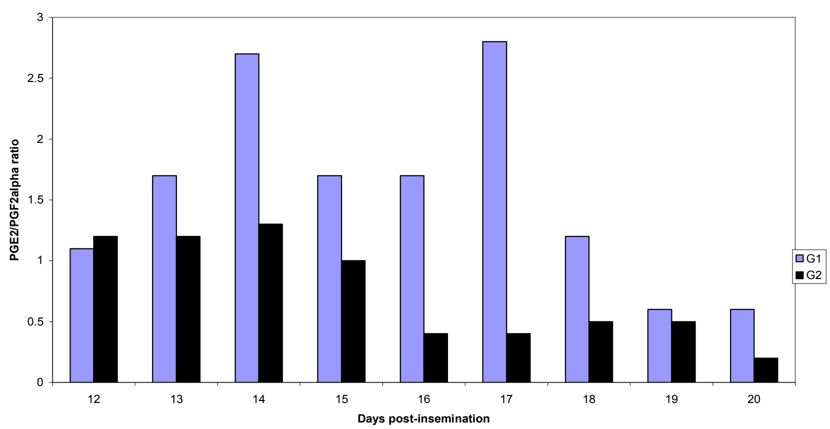

Figure 4: PGE2/PGF2alpha ratio in pregnant (G1) and non-pregnant (G2) cows during the period from day 12 to day 20 post-insemination

DISCUSSION

The present findings of P_4 concentration in pregnant and non-pregnant Friesian cows (Figure 1) agree with the findings of Oyedipe *et al.* (1986), Peters (1996) and Royal *et al.* (2000). The high level of progesterone in pregnant cows is due to the maintenance of corpus luteum (CL) (Peters, 1996; Mann *et al.,*1999 and Niswender *et al.,* 2000) to support pregnancy (Spencer and Bazer, 2002 and Inskeep, 2004). Maintaining CL is mainly due to the secretion of interferon-tau (IFN-τ) by the embryos which inhibits the mechanism of luteolysis of CL (Peters,1996).

Increasing $PGF_{2\alpha}$ concentration in non-pregnant *vs.* its decrease in pregnant around days 16-17 (maternal recognition period) (Figure 2) is in accordance with the findings of Bazer *et al*.(1991) and Okuda *et al*.(2002). Secretion of IFN-τ, inhibits enzyme(s) necessary for $PGF_{2\alpha}$ synthesis (Bazer *et al.,*1991).

High concentration of $PGF_{2\alpha}$ in non-pregnant cows is most probably attributed to the direct effect to increase oxytocin from uterine endometrium (Peters and Ball, 1995 and Peters, 1996). Increase of Oxytocin in non-pregnant cows is due to the increase of estradiol-17β concentration (Inskeep,2004) accompanying the growth of new follicles.

Pattern of PGE_2 secretion agrees with that of Asselin *et al.* (1997); Parent *et al*. (2002) and Inskeep (2004) who reported that PGE_2 concentration is higher in pregnant than in non-pregnant animals. Stimulation of PGE_2 secretion may be a response to the secretion of IFN-τ which acts as the mechanism of maternal recognition in bovine. The obtained ratio PGE_2/ $PGF_{2\alpha}$ (Figure 4) is in agreement with the studies of Parent *et al.* (2002) and Inskeep (2004).

The increase in PGE_2 in G_1 is due to the rapid increase of of cyclooxygenase (COX-2) enzyme (Parent *et al.,* 2002) as a result of IFN-τ increase. The enzyme inhibits the conversion of PGE_2 to $PGF_{2\alpha}$ by a putative 9-Keto-prostaglandin E_2 (Asselin and Fortier, 2000) and may transform the response of endometrium to oxytocin from the stimulation of $PGF_{2\alpha}$ to the stimulation of PGE_2 (Asselin *et al.*,1997).

However, while this study arrived at a description of hormonal balance during summer season, another study is needed to compare between the levels of hormone during winter and summer seasons.

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النموذج الهرموني المصاحب لثبوت الحمل فى أبقار الفريزيان وعلاقته بظروف موسم الصيف في مصر

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أجريت هذه الدراسة على ١٦ بقرة فريزيان متعددة الولادات تم تلقيحها خلال موسم الصيف (يونيه – أغسطس) وذلك لمقارنة النموذج الهرموني خلال فترة ثبوت الحمل (تعرف الأم على الحمل) للأبقار التي حدث بها حمل والأخرى التي لم يحدث بها حمل.

تم ملاحظة الشياع ثلاث مرات يوميا وتم تلقيح مجموعة من الأبقار التي ثبت شيا عها بالسائل المنوي المجمد بعد ٨–١٦ ساعة من بداية الشياع. تم تجميع عينات الدم يوميا ابتداء من يوم التلقيح حتى اليوم ٢١ بعد التلقيح لتحديد مستوى هرمون البروجستيرون بينما تم تحديد مستوى تركيز البروستاجلاندين $PGF_{2\alpha}$ والبروستاجلاندين PGE_2

خلال فترة التعرف على الحمل (من اليوم ١٢ إلى اليوم ٢٠ بعد التلقيح). تم تشخيص الحمل بالجس المستقيمى في اليوم ٤٥ بعد التلقيح ونبعا لنتائج الجس المستقيمى تم الأبقار إلى مجموعتين (٨ أبقار لكل مجموعة) الأولى تضم الأبقار العشار والثانية تضم الأبقار الغير عشار.

أظهرت النتائج أن أبقار المجموعة الأولى كانت ذات مستوى أعلى فى البروجستيرون والبروستاجلاندين PGE_2 من أبقار المجموعة الثانية بينما كان مستوى البروستاجلاندين $PGF_{2\alpha}$ اقل فى المجموعة الأولى عن الثانية خلال فترة التعرف على الحمل (اليوم١٢ حتى اليوم ٢٠) وكانت النسبة بين البروستاجلاندين $PGF_{2\alpha}$ و البروستاجلاندين PGE_2 فى اليوم ١٦ واليوم ١٧ كانت ٤.٢ و٧ اضعاف في المجموعة الأولى عن الثانية على التوالي.