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Effect Of Gnrh Administration At Mating And During Luteal Phase On Pregnancy Rate Of Repeat Breeder Buffalo Cows

Abo-Farw, M.A.* and M.M. Ghoneim*
*Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.

Abstract

To evaluate the potentiality of GnRH agonist injection, on day 0, 11 or 13 post-mating, on pregnancy rate of repeat breeder buffalo cows, total of 40 cyclic Egyptian buffalo cows (4-7 years and 400-500 kg weight) were i.m. injected with 2.5 ml PGF2α to synchronize estrus. Animals in heat (within 72 h) were naturally mated and allotted randomly to four groups (10 in each). Animals in the 1st, 2nd and 3rd groups were i.m. injected with 2.5 ml GnRH on day 0 (G1), 11 (G2) and 13 (G3) of estrus onset, respectively, while G4 was control injected with 2.5 ml saline. Blood samples were collected from jugular vein on day 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 of estrus. Pregnancy was diagnosed on day 25 post-mating of each animal using ultrasound examination. Results showed that serum P_4 concentration increased ($P<0.05$) following GnRH injection up to day 13, 17 and 25 post-mating in G1, G2 and G3 as compared to G4, respectively. Serum P4 was the highest ($P<0.05$) in G3 from day 17 to 25 post-mating, being different ($P<0.05$) from G4, but did not differ than that in G1 and G2. Pregnancy rate was 70, 70, 80 and 50% in G1, G2, G3 and G4, respectively ($P<0.05$). Concentration of P4 was higher ($P<0.05$) in pregnant than in non-pregnant buffaloes as overall mean for all groups or within each experimental group, being the highest in G3.

Therefore this study may conclude that injection of GnRH analogue (2.5 ml Receptal/animal) on day 13 post-mating improved serum progesterone concentration and pregnancy rate of repeat breeder buffalo cows as compared to those injected on day 0 or 11 post-mating.

Keywords: Buffalo, repeat breeder, GnRH, estrous, luteal phase, progesterone, pregnancy.

Introduction

Reproductive efficiency is the primary factor affecting productivity which is hampered in buffalo cows by inherent late maturity, poor estrous expression, distinct seasonal reproductive pattern and prolonged calving intervals (Singh et al. 2000). Repeat breeding in dairy cows is associated with estrus detection error, endocrine dysfunctions, ovulation defects, uterine infection, gamete quality, etc., and therefore, poor fertilization rate and/or early embryonic death (Patel, et al., 2014).

Early embryonic mortality (EM) is one of a major economic loss to dairy producers. In bovine, approximately 25% of embryos are lost in the first 3 weeks of life (Peters, 1996) of which the greatest proportion seems to occur between 14 and 17 days after ovulation (Sreenan et al., 2000). Majority of EM in cattle occurs during pre-implantation stage, i.e. during the first 20 days of pregnancy, when 75-80% of fertilized eggs are lost (Bullman and Laming, 1978).

It was mentioned that EM may be, partially, attributed to a decrease in progesterone (P4) secretion by corpus luteum (CL) during early pregnancy (Campanile et al., 2005) and elevated concentrations of circulating P4 are required for conceptus development in repeat breeding crossbred cattle (Savalia et al., 2014). More exposure to P4 by the embryo may increase its chance of secreting interferon-t and thus survive (Thatcher et al., 2001). Abnormal CL function in early and mid-luteal phase of estrous cycle results in low P4 production in peripheral circulation, which may cause early EM and reducing pregnancy rate (Hommeidaa et al., 2004). Therefore, further injection of GnRH during mid-luteal phase after insemination induces sufficient release of LH and FSH to increase the lifespan of CL by counteracting luteolysis through disruption of normal follicular growth and secretion of estrogen, thereby permitting maternal recognition of pregnancy to occur (Willard et al. 2003).

Many methods have been tried to increase conception rate (Beltran and Vasconcelos, 2008) and to reduce EM (Gaja et al., 2008) by enhancing endogenous P4 level as lower than the normal rise and lower total P4 concentration have been reported in repeat breeder cows. This can be achieved by inducing the formation of accessory CL, which can be obtained by GnRH treatment on day 4-6 post-AI (Kulasekar, et al., 2012), but GnRH effects on pregnancy rates and plasma P4 profile are inconsistent. Therefore, use of GnRH or its agonists to increase conception rates should be based on an understanding of GnRH-induced biological effects on the reproductive-endocrine system. This effect may occur through GnRH-stimulated LH surge, stimulating P4 production by CL (Gaja et al., 2008). In buffaloes, treatment with GnRH agonist on day 5 after AI induced ovulation rate of 62% and increased milk whey P4 in response to gonadotrophin (Campanile et al., 2007a). Buserelin or hCG treatment increased P4 level but did not

reduce the EM, with injection on day 5 after AI (Campanile et al., 2007b) and increased P4 level and reduced EM in Mediterranean buffaloes with administration on 25 days after AI (Campanile et al., 2007c). Recently, conception rate of buffaloes improved by GnRH agonist administration on day 13 post-breeding compared to day 0 or day 11 probably due to its beneficial effect on embryo survival by enhancing luteal function (Attoo et al., 2013).

In lactating dairy cattle, injections of GnRH agonist (buserelin) between days 11 and 13 after insemination resulted in extended inter estrous intervals, elevated serum P4 levels and remarkable improvement in pregnancy rate (Rettmer et al. 1992; Stevenson et al. 1993; Drew and Peters, 1994; peters et al., 2000).

Therefore, the present study aimed to evaluate the potentiality of GnRH agonist injection, on day of mating (0 day) or during different days post-mating (Day 11 or 13), on pregnancy rate of repeat breeding buffalo cows

Materials and methods

This study was conducted at Animal Production Experimental Station, Mehallet Moussa, Kaferelsheikh Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture during the period from April 2014 to August 2014.

Animals:

A total of 40 cyclic Egyptian buffalo cows (4-7 years old and 400-500 kg live body weight) was used in this study. All experimental animals had a history of normal calving. All buffalo cows used in this study showed previously normal estrous cycle and had no clinically illness signs detectable. The examination of reproductive tract of all buffaloes by rectal palpation and ultrasonography revealed that the genital tract of all animals were free from any pathological diseases and disorders. All buffaloes failed to conceive after more than 3 services per animal to be considered as repeat breeder buffalo cows. All animals had mature CLs at the beginning of the experimental period.

The experimental animals were randomly allocated to four groups according to the hormonal treatment (10 animals in each). Through the experimental period, all experimental animals were kept under the regular systems of feeding and management adopted by Animal Production Research Institute. Fresh water was available all times. Buffalo cows were housed in semi-open sheds.

Experimental design:

All experimental animals had mature CL and each animal was injected i.m. with 2.5 ml of PGF2α analogue (Estrumate, Essex Animal Health Fresoythe Sedelsberger Strasse 2-4. 26169 Friesoythe, Germany) to synchronize the estrus in all experimental animals. Each ml Estrumate contained 0.625 µg/ml Cloprostenol sodium. Estrous activity was detected within 72 h after PGF2α injection at every morning and evening by close observation for external signs in presence of a teaser buffalo bull all the times of observation.

Buffaloes in heat were naturally inseminated by fertile buffalo bull and allotted randomly to four experimental groups (10 animals in each). Buffaloes in the 1st, 2nd and 3rd groups were i.m. injected with a single dose of 2.5 ml GnRH analogue (Receptal, Product of Intervet International GmbH. Germany, Imported by Intervet Egypt. Reg N 2139) on day 0 (G1), 11 (G2) and 13 (G3) of estrus onset, respectively. Each ml Receptal contained 4µg Buserelin acetate. However, buffalo cows in the 4th group (G4) were injected with 2.5 ml saline at the time of mating and were considered as control group.

Blood samples:

Blood samples were collected by jugular vein puncture from all animals of each group on days 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 of mating. Blood samples were collected in sterilized glass tubes and kept at room temperature. Within an hour after collection, samples were centrifuged at 3000 rpm for 15 min, then serum was collected and transferred into sterilized vials. All serum samples were stored at -20 °C to for determination of P4 concentration in blood serum.

Hormone assay:

Direct Radioimmunoassay technique (RIA) was performed for determination of serum P4 concentration using ready antibody coated tubes kit (Diagnosis Systems Laboratories Texas, USA) according to the procedure outlined by the manufacturer.

Pregnancy diagnosis:

Pregnancy was diagnosed on day 25 post-mating of each animal using ultrasound examination (Digital ultrasonic diagnostic imaging System, Model Dp-30 Vet. 50/60 HZ, SHENZHEN, MINDRAY BIO-MEDICAL.ELECTRONICS, CO. LTD) 7.5 MHz Linear array transducer and Depth 4.3. Pregnancy was indicated by rectal palpation of in non-returned animals on day 45-50 post-mating. Then pregnancy rate was calculated.

Statistical analysis:

Statistical analysis of the obtained data was performed using general linear model of SAS (2000). The significant differences among treatment means were performed using Duncan's Multiple Range Test (Duncan, 1955).

Results and discussion

Progesterone profile:

Effect of GnRH treatment on serum P4 concentration of buffalo cows was significant ($P<0.05$) at all sampling time, except on day 0 and 17 post-mating. Serum P_4 concentration significantly ($P<0.05$) increased following GnRH injection up to day 13, 17 and 25 post-mating in G1, G2 and G3 as compared to G4 (control), respectively (Table 1).

It is of interest to note that serum P4 concentration pre-GnRH injection was significantly ($P<0.05$) higher in G3 than in G1, but did not differ from that in G1 treated with GnRH on 0 day post-mating. Such trend in P4 concentration may be related to increasing number of pregnant animals in G3 (8 out of 10 animals) as compared to G4 (5 out of 10 animals). In addition, serum P4 from day 17 up to 25 post-mating was the highest in G3, being significantly ($P<0.05$) different from G4 (control), but did not differ significantly than that in G1 and G2. However, the differences in P4 concentration during the same period among both G1 and G2 and control (G4) were not significant (Table 1).

These results indicated elevated P4 concentration following GnRH injection in each treatment group, but GnRH injection resulted in the best results up to 25 day post-mating.

Table 1: Progesterone (P_4) concentration (ng/ml) in blood serum of buffalo cows in the experimental groups.

Post-mating day	Day of GnRH treatment			G4 (Control)	Significance
	G1(0)	G2 (11)	G3 (13)		
0	0.597±0.056	0.551±0.050	0.572±0.056	0.515±0.048	NS
3	1.346±0.067^{a}	0.801±0.076^{b}	0.827±0.081^{b}	0.771±0.047^{b}	*
5	2.133±0.139^{a}	1.679±0.117^{b}	2.179±0.148^{a}	1.272±0.068^{c}	*
7	3.420±0.192^{a}	2.865±0.174^{b}	3.659±0.139^{a}	2.852±0.144^{b}	*
9	4.278±0.215^{a}	3.708±0.259^{ab}	4.201±0.278^{a}	3.226±0.242^{b}	*
11	4.215±0.153^{a}	4.050±0.165^{ab}	3.857±0.190^{ab}	3.509±0.265^{b}	*
13	4.385±0.168^{a}	4.783±0.135^{a}	4.287±0.259^{ab}	3.659±0.311^{b}	*
15	4.469±0.387^{b}	5.541±0.137^{a}	5.285±0.240^{a}	4.408±0.210^{b}	*
17	4.959±0.547	5.137±0.408	5.633±0.432	4.458±0.469	NS
19	4.477±0.537^{b}	5.188±0.545^{ab}	6.147±0.464^{a}	4.318±0.486^{b}	*
21	4.757±0.706^{ab}	4.140±0.774^{ab}	6.101±0.886^{a}	3.534±0.913^{b}	*
23	4.649±0.937^{ab}	4.317±0.855^{ab}	6.463±0.973^{a}	3.626±0.869^{b}	*
25	4.088±0.797^{ab}	5.209±0.761^{ab}	6.287±0.844^{a}	3.355±0.726^{b}	*

a, b and c: **Means denoted within the same row with different superscripts are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05.**

Results showed insignificant differences in serum P_4 on day of estrus, being ≤0.5 ng/ml in all groups, which may indicate incidence of estrus and ovulation as well as complete regression of all CLs presented at the beginning of treatment in all experimental groups. It is well known that all animals used in this study were treated with PGF2α to induce estrus/ovulation of treated buffalo cows. Many authors reported similar findings on Egyptian buffalo cows (El-Moghazy et al., 2006) and buffalo heifers (Aboul-Ela et al., 2006). Also, there is a remarkable increase in P4 concentration in treated groups up to 25 days post-mating as a result of CL formation of pregnancy, while marked reduction was observed in G4 (control) after day 19 of mating due to increasing number of non-pregnant animals (at the end of luteal phase).

The present results are in agreement with Attoo et al. (2013), who showed significant ($P<0.05$) differences in serum P4 levels in buffaloes treated with GnRH on day 5 (1.78 ng/ml), day 13 (4.51 ng/ml), and day 18 (6.55 ng/ml) post-mating in comparison with control group on the same days (1.39, 3.77 and 5.11 ng/ml, respectively).Also, the increase in P4 concentration as affected by GnRH injection was noted in dairy heifers (Karimi et al., 2007), in Indian buffaloes (Mandal et al., 2009) and Egyptian buffalo heifers (Abo-Farw et al., 2016).

The noted elevation of P4 concentration in GnRH treated animals may be due to that injection of GnRH stimulates the transformation of follicular cells to luteal cells, which was required at least 2 to 3 days for optimum P4 production (Stevenson et al., 1993). Treatment of GnRH at estrus may potentiate

conversion of small luteal cells to large luteal cells resulting into development of large sized functional CL required for embryo survival through enhanced P4 secretion.

Pregnancy rate:

Pregnancy rate of buffalo cows was affected significantly (P<0.05) by GnRH treatment, being the highest in G3 (80%), moderate in G1 and G2 (70%) and the lowest in G4 (50%). This finding indicated a beneficial effect of GnRH injection on days 0, 11 and 13 post-mating on pregnancy rate of repeat breeder buffalo cows, but GnRH injections on day 13 of synchronized estrous cycle was more effective on improving CR as compared to that injected at the time of estrus and day 11.

It is worthy noting that pregnancy rate in each experimental group is in association with P4 concentration. Failure or delay of ovulation might be prevented and conception rate might increase by GnRH administered at insemination. In accordance with the present results, pregnancy rate was significantly improved in Egyptian buffalo heifers (Abo-Farw et al., 2016) and Indian buffaloes (Attoo et al., 2013) when animals were treated with GnRH on day of estrus as compared to control. Also, Mandal et al. (2009) reported an improvement in pregnancy rate of buffaloes treated with GnRH on day of estrus as compared to control (87.5 vs. 75.0%). Furthermore, An improvement of about 33% in conception rate was recorded in buffaloes treated with GnRH analogue during days 11-13 after insemination.

Table 2: Pregnancy rate (%) of buffalo cows in the experimental groups.

Post-mating day	Day of GnRH treatment			G4 (Control)
	G1(0)	G2 (11)	G3 (13)	
Number of animals	10	10	10	10
Pregnant animals	7	7	8	5
Non pregnant animals	3	3	2	5
Pregnancy rate (%)	70[b]	70[b]	80[a]	50[c]

a, b and c: **Means denoted within the same row with different superscripts are significantly different at P<0.05.**

In cattle, Iftikhar et al. (2009) observed improvement in pregnancy rate (37.5 vs. 68.75%) when GnRH was injected at the time of insemination. Karimi et al. (2007) showed 10% improvement in conception rate (70% vs. 80%) when GnRH was administered to dairy heifers on day of estrus. Rangnekar et al. (2002) reported pregnancy rate of 70% in repeat breeder Holstein-Friesian cows administrated with GnRH. The improvement in pregnancy rate of buffalo cows treated with GnRH on day of estrus observed in our study may possibly be related to better synchrony of preovulatory LH surge and ovulation (Tanabe et al., 1994; Yoshioka et al. 2001), due to its beneficial effect on embryo survival by enhancing luteal function (Attoo et al., 2013), or by stimulating the transformation of follicular cells to luteal cells, which was required at least 2 to 3 days for optimum P4 production (Stevenson et al., 1993). GnRH at estrus may potentiate conversion of small luteal cells to large luteal cells resulting into development of large sized functional CL required for embryo survival through enhanced P4 secretion (Attoo et al., 2013).

Concerning the effect of time of GnRH treatment, Abo-Farw et al. (2016) in Egyptian buffalo heifers Attoo et al. (2013) in Indian buffaloes found that pregnancy rate significantly improved when animals were treated with GnRH on 13 day post-mating as compared to those treated on 0 or 11 day post-mating. Also, Mandal et al. (2004) showed an improvement in the conception rate of buffaloes (75% vs. 45%) treated with GnRH on day 12 of estrous cycle compared to control at first insemination.

Poor fertilization rate and/or early embryonic mortality are considered as major factors affecting pregnancy rate of dairy cows (Patel, et al., 2014). This may be due to marked reduction in P4 concentration produced from CL during the 1st three weeks post-mating (Campanile et al., 2005). In our study, repeat breeder buffalo cows may have CL dysfunction and low P4 level and consequently may cause early embryonic death, reflecting low pregnancy rate (Hommeidaa et al., 2004). Further treatment of repeat breeder buffalo cows with GnRH during mid-luteal phase (on 11 or 13 day post-mating) in the present study may increase concentrations of circulating P4 required for pregnancy maintenance (Savalia et al., 2014). Also, GnRH injection during mid-luteal phase may cause sufficient release of LH and FSH to increase the life span of CL by counteracting luteolysis through disruption of normal follicular growth and secretion of estrogen, thereby permitting maternal recognition of pregnancy to occur (Willard et al. 2003).

On the other hand, other studies reported that GnRH administration failed to exhibit a positive impact on pregnancy rate of beef cattle (Perry and Perry, 2009), cows (Anderson and Malmo, 1985) or repeat breeder cows (Young and Swanson, 1988). A reason for non-impact on fertility could be due to

GnRH-induced ovulation of physiologically immature follicles that had a negative impact on pregnancy rates and lead to late embryonic/fetal survival (Busch et al., 2008; Lynch et al., 2010).

Progesterone profile in pregnant and non-pregnant buffalo cows:

Overall mean of P4 concentration for all groups for all sampling times was significantly ($P<0.05$) higher in pregnant than in non-pregnant buffaloes (4.33±0.093 vs. 2.29±0.108 ng/ml), being almost higher in pregnant than in non-pregnant animals within each group (Fig. 1).

Several authors reported that animals were regarded to be pregnant when the P4 concentration in serum was ≥1.6 ng/ml on day 24 post-mating in buffaloes (El-Moghazy, 2003) and cows (Yildiz et al., 2009). Results illustrated in Fig. 1 revealed also that P4 concentration of pregnant animals was higher in treated groups than in control, being the highest in G3 treated with GnRH on 13 day post-mating.

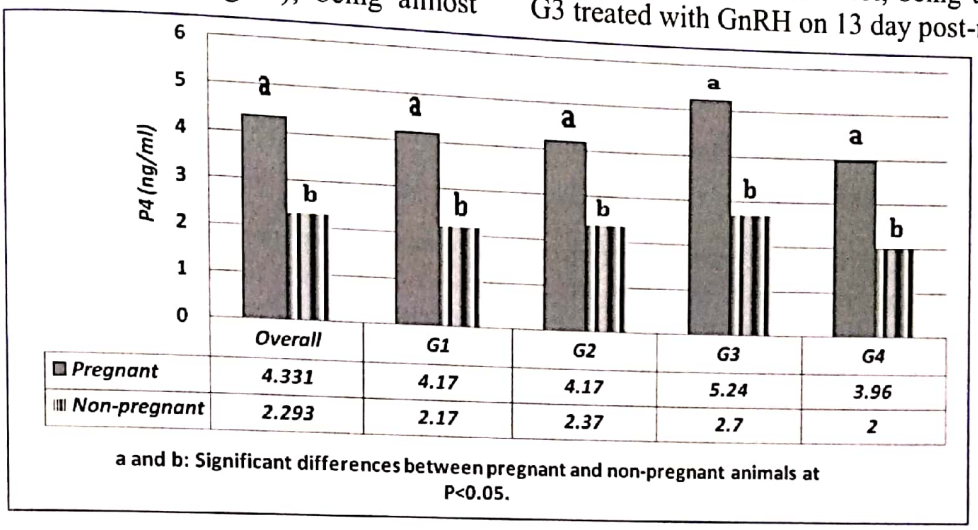

Fig. 1: Overall mean of P4 concentration (ng/ml) in blood serum of pregnant and non-pregnant buffalo cows in all groups and within each group.

In consistent with the present results, it was reported significant increase in P4 from day 8 to day 22 when GnRH was given on day of estrus in buffaloes (Mandal et al., 2009) and repeat breeder cows (Selvaraj and Kumar, 2001). In addition, many authors showed that injection of GnRH on days 11 to 14 after AI in lactating cows increased serum P4 level (Tefera et al., 2001; Howard et al., 2006). The increased P4 concentration in GnRH treated cows could be explained by firstly that the GnRH induces an additional LH surge to enhance active luetinization of granulose and theca cells to ensure adequate production of P4 in developing CL. Secondly, GnRH may have acted on the developing CL to promote the conversion of small luteal cells to large luteal cells, which are responsible for about 85% of basal progesterone secretion at luteal phase (Niswender et al. 1985). Administration of GnRH at estrus may induce the release of both LH and FSH in buffaloes which cause maturation of ovarian follicles and ovulation. This might also act by enhancing or altering theca lutein cells in the preovulatory and postovulatory follicles or on developing CL to promote conversion of small lutein cells into large lutein cells, resulting into development of large sized functional CL, enhancing P4 secretion required for embryo survival (Aboul-Ela et al., 1985). Administration of GnRH during any stage of the estrous cycle leads to an LH surge (Campanile et al. 2008; Yildiz et al. 2009). Moreover, GnRH treatment may be utilized in order to induce the formation of an accessory CL and hence, to increase P4 levels, which are critical for maintaining the pregnancy (Campanile et al., 2007).

Concerning the overall mean of P4 concentration throughout sampling days, the significant ($P<0.05$) superiority of pregnant animals appeared from 19 up to 25 day post-mating up to (Fig. 2). In the present study, overall mean of P4 concentration on 23 and 25 day post-mating was 0.785±0.158 and 1.314±0.123 ng/ml in non-pregnant versus 6.679±0.218 and 6.381±0.247 ng/ml in pregnant buffaloes.

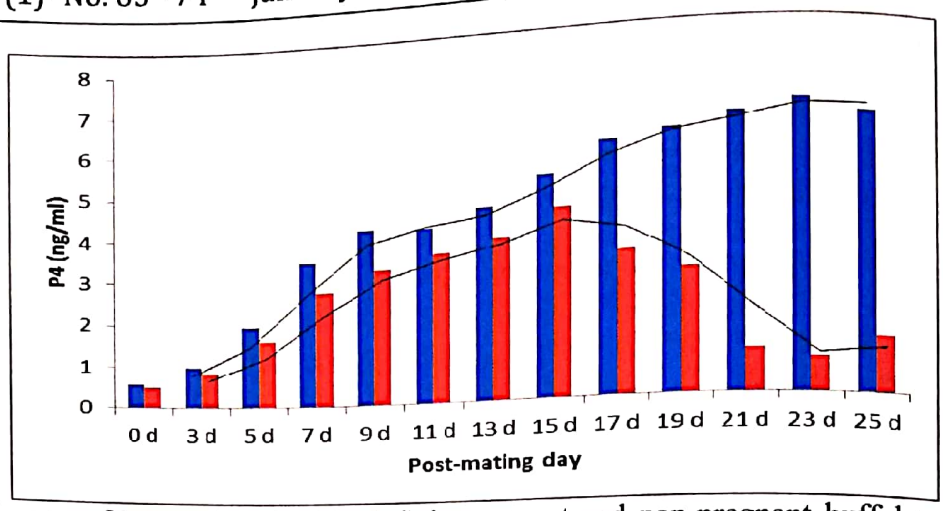

Fig. 2: Overall mean of P4 concentration (ng/ml) in pregnant and non-pregnant buffalo cows throughout different sampling days.

When P4 concentration was compared in pregnant and non-pregnant animals at each sampling time, results illustrated in Fig. 3 revealed that P4 concentration in blood serum of buffalo cows was nearly similar up to day 13 in G1 and on day 15 post-mating in other groups, then P4 level continued to increase in pregnant animals as a result of presence of functional CL, while showed sharp reduction due to regression of CL and end of the luteal phase in non-pregnant animals up to day 23 post-mating.

Conclusion

Results obtained in the current study revealed remarkable effect of GnRH on day 0, 11 or 13 post-mating on P4 profile and pregnancy rate of repeat breeder buffalo cows. Therefore this study may conclude that injection of GnRH analogue (2.5 ml Receptal/animal) on day 13 post-mating improved serum progesterone concentration and pregnancy rate of repeat breeder buffalo cows as compared to those injected on day 0 or 11 post-mating.

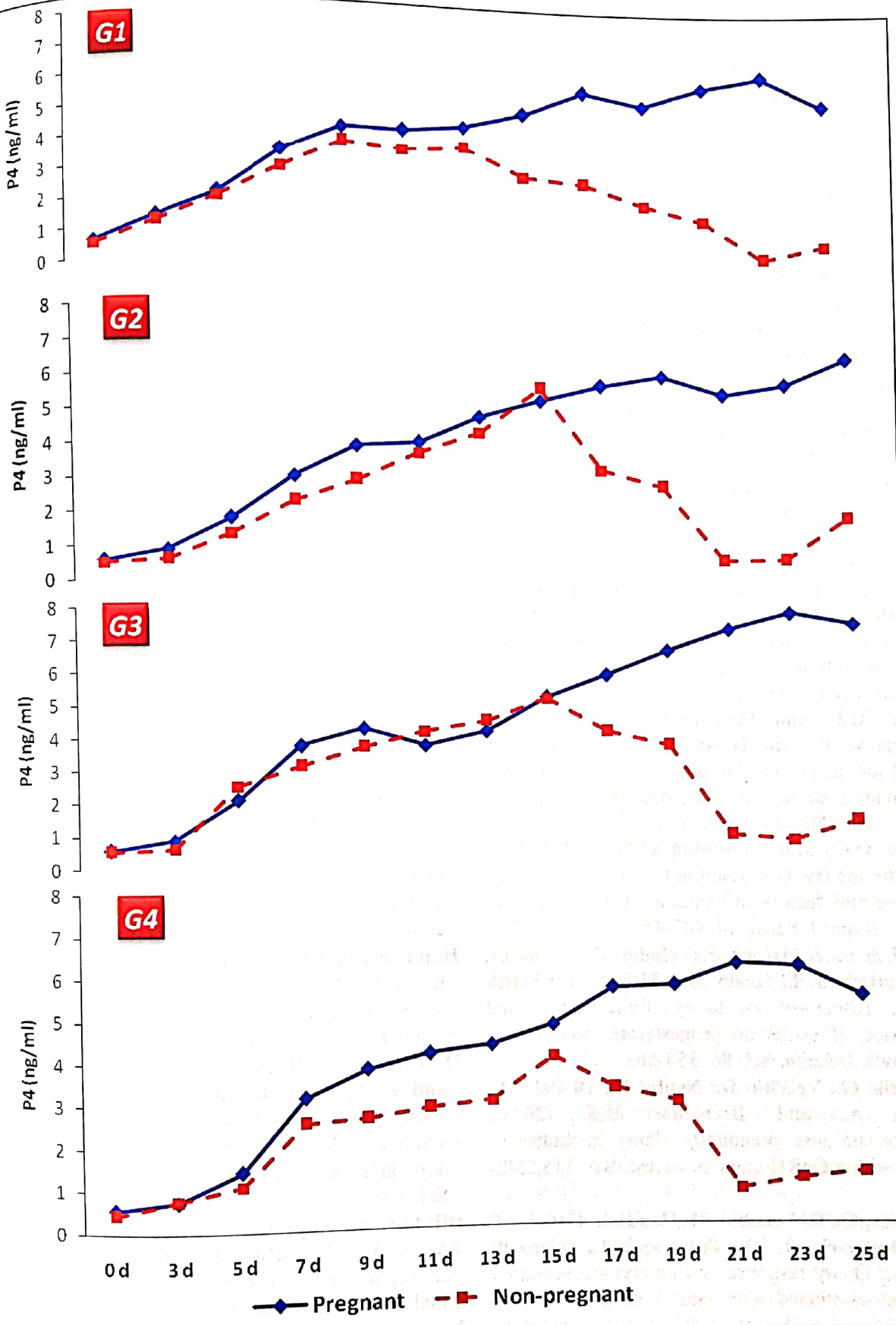

Fig. 3: Concentration (ng/ml) of P4 in pregnant and non-pregnant buffalo cows in each experimental group throughout different sampling days.

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الملخص العربي

تأثير المعاملة بالهرمون المنشط لهرمونات الغدد الجنسية عند التلقيح وأثناء مرحله سيادة الجسم الاصفر على معدلات الحمل في إناث الجاموس متكرر التلقيح .

محمد عبد الفتاح ابو فرو* - مسعود محمد غنيم*

*معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية

هدف هذه الدراسة الى تقييم كفاءة الحقن بالهرمون المنشط لإفراز هرمونات الغدد الجنسية (GnRH) في ايام صفر و ١١ و ١٣ من التلقيح على معدل الحمل في إناث الجاموس متكرر التلقيح، استخدم في هذه الدراسة عدد (٤٠) من إناث الجاموس المصري متكررة التلقيح (لم تحسب بعد ثلاث تلقيحات على الاقل) عمرها من ٤-٧ سنوات ويتراوح وزنها من ٤٠٠-٥٠٠ كجم و تم تقسيمها عشوائيا الى ٤ مجموعات كل مجموعة تحتوى على ١٠ حيوانات، تم حقن جميع الحيوانات والمحتوية على أجسام صفراء ناضجة بـ٢.٥مل بروستاجلاندين (استروميت) كل حيوان لعمل تزامن شبقي.

تم تلقيح الحيوانات التي اظهرت شياع (بعد الحقن بالبروستاجلاندين) خلال ٧٢ ساعة في كل المجاميع طبيعيا بفحل ناضج جنسيا وفى يوم شياع تم حقن الحيوانات في المجموعة الاولى و الثانية والثالثة بـ ٢.٥ مل ريسبتال لكل حيوان في اليوم صفر و ١١ و ١٣ من بداية الشياع على التوالي بينما المجموعة الرابعة كانت ضابطه وتم حقنها بمحلول ملح فسيولوجي ٢.٥ مل لكل حيوان. تم جمع عينات الدم في اليوم صفر ٣،٥،٧،٩،١١،١٣،١٥،١٧،١٩،٢٣،٢٥ من الشياع لتقدير تركيز البروجيستيرون في مصل الدم، كذلك تم تشخيص الحمل في كل حيوان بعد ٢٥ يوم من التلقيح باستخدام جهاز الموجات فوق الصوتية وقد اوضحت النتائج ما يلي:

١. ارتفع معدل تركيز البروجيستيرون في مصل الدم معنويا ($P<0.05$) نتيجة الحقن ب GnRH حتى اليوم ١٣ ،١٧ و ٢٥ بعد التلقيح فى المجموعة الاولى والثانية والثالثة بالمقارنة بالمجموعة الرابعة الضابطة على التوالي .

٢. كان تركيز البروجيستيرون الاعلى معنويا ($P<0.05$) في المجموعة الثالثة من اليوم ١٧ حتى اليوم ٢٥ بعد التلقيح وكان الاختلاف معنويا ($P<0.05$) عن المجموعة الرابعة ، ولم يختلف معنويا عن المجموعة الاولى والثانية .

٣. كانت معدلات الحمل ٧٠ ,٧٠ ,80 ,٥٠% في المجموعة الاولى والثانية والثالثة والرابعة على التوالي وكانت الاختلافات معنوية ($P<0.05$).

٤. كان تركيز البروجيستيرون أعلى معنويا ($P<0.05$) في إناث الجاموس العشار عن الغير عشار كمتوسط عام لكل المجاميع او داخل كل مجموعة وكانت زيادة البروجيستيرون اوضح في المجموعة الثالثة .

يستخلص من هذه الدراسة ان المعاملة بالهرمون المنشط لإفراز الهرمونات الجونادوتروفينيه (٢.٥مل ريسبتال/حيوان) في اليوم ١٣من شياع/التلقيح أدى الى تحسين تركيز البروجيستيرون في مصل الدم وتحسين معدل الحمل في إناث الجاموس المصري متكررة التلقيح المقارنة مع تلك التي تم حقنها في اليوم صفر واليوم ١١ من التلقيح او المجموعة المقارنة.