EFFECT OF GnRH TREATMENT DURING DIFFERENT TIMES POST-MATING ON PREGNANCY RATE OF BUFFALO HEIFERS

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SUMMARY

To evaluate the efficiency of GnRH injection, on day of mating (Day 0) or during different days post-mating (Day 10 or Day 12),on conception rate of repeat breeder buffalo heifers, a total of 20 cyclic Egyptian buffalo heifers (2-4 years of age and 350-400 kg LBW was allotted to four experimental groups. All heifers had mature CLs and were injected i.m. with 2.5 ml PGF2α analogue (Estrumate)/h. Heifers in heat were naturally mated by fertile buffalo bull and allotted randomly to four experimental groups, 5 animals in each. On day of estrus, heifers were injected with 2.5 ml saline (G1, control), or a single dose of 2.5 ml Receptal on days 0, 10 and 12 post-mating in G2, G3 and G4, respectively. Blood samples were collected for determination of P4 concentration in blood serum. Pregnancy was diagnosed on day 25 post-mating of each animal using ultrasound examination. Results showed that conception rate (CR) was the highest (P<0.05) in G3 and G4 (80%), moderate in G2 (60%) and the lowest in G1 (40%). Serum P4 concentration was the highest (P<0.05) in G4 on most sampling days. There were insignificant differences in serum P4 on day of estrus, being ≤0.5 ng/ml in all groups. Concentration of P4 was the highest (P<0.05) in G2 as compared to other groups. Heifers in G4 showed the highest response to GnRH injection on day 12, whereas P4 concentration tended to increase on day of GnRH injection. There were marked group differences in P4 concentration on days 22 and 24, but P4 concentration was always ≥2 ng/ml in all groups. Level of P4 was higher (P<0.05) in pregnant than in non-pregnant heifers within each group.

In conclusion, GnRH administration (2.5 ml Receptal/h) on day 12 of estrus/mating had positive effect on pregnancy rate of repeat breeder buffalo heifers as compared to those injected on day 0 and 10 of mating.

Keywords: buffaloes, repeat breeder, GnRH, conception rate, progesterone

INTRODUCTION

The success of dairy industry is dependent on the milk production of dairy cattle and buffaloes (Bajaj *et al*., 2006).Milk production is directly related to the reproductive phenomenon. Mortality is more common during the early embryonic period (from day 8^{th} to 16^{th}) without an effect on estrous cycle length (Bajaj and Neelesh, 2011)and it is one of the major causes for repeat breeding in dairy cows (Santos*et al*., 2004 and Diskin and Morris, 2008), leading to declined fertility in buffaloes.Embryonic mortality (EM) may be, partially, attributed to a decrease in progesterone (P_4) secretion by the corpus luteum (CL) during early pregnancy (Campanile *et al*., 2005). EM appears to occur on day 25-40 in buffalo (Campanile *et al*., 2005) later than on day 14-17 in cows (Mann, 2002).

The GnRH was suggested to have luteotropic and luteoprotective factors, thereby enabling maternal recognition of pregnancy (Macmillan *et al*., 1986). Abnormal CL function is associated with reduced pregnancy rates (Hommeidaa *et al*., 2004). Measurement of blood P_4 suggested that embryonic death at the time of CL maintenance delayed luteolysis and interestrus interval (Humblot, 2001). GnRH treatment may increase the chances of embryo survival by improving luteal function and/or interfering with the luteolytic mechanism (Beck *et al*., 1994; Birnie *et al*., 1997 and ; Cam *et al*., 2002). Several hormonal treatments have been attempted to reduce embryonic loss or improve pregnancy rates in cattle, including the use of gonadotrophin releasing hormone (GnRH) and human chorionic gonadotropin (HCG), but their effects on pregnancy rates and plasma P4 profile are inconsistent(Yildiz *et al*., 2009).

In lactating dairy cows, injection of GnRH agonist (buserelin) between the 11^{th} and 13^{th}day post-mating resulted in extended inter-estrous interval and elevated level of P+ in blood serum (Stevenson *et al.*, 1993). GnRH-induced biological affects the reproductive-endocrine system to increase conception rates. This effect may induce through GnRH-stimulated LH surge and stimulating P_4 production by CL (Gaja *et al*., 2008).In buffalo, 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 P_4 level but did not reduce the EM, with injection on day 5 after AI (Campanile *et al*., 2007b) and increased P_4 level and reduced EM in Mediterranean buffaloes with administration on 25 days after AI (Campanile *et al*., 2007c).

The objective of the this study was to evaluate the potentiality of GnRH injection, on day of mating (0 day) or during different days post-mating (Day 10 or 12), on pregnancy rate of repeat breeder buffalo heifers.

MATERIALS AND METHODS

The 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 June 2015.

Animals:

A total of 20 cyclic Egyptian buffalo heifers, 2 to 4 years of age and 350-400 kg live body weight was used in this study. The cyclicity of heifers was proved by mating these heifers more than three times with returning to estrus. All heifers used in this study showed previously normal estrous cycle and had no clinically illness signs detectable. The examination of reproductive tract of all heifers by rectal palpation and ultrasonography revealed that the genital tract of all animals were free from any pathological diseases and disorders. All heifers failed to conceive after more than 3 services per animal to be considered as repeat breeder buffalo heifers. All animals had mature CLs at the beginning of the experimental period.

The experimental animals were allotted to four experimental groups according to the hormonal treatment (5 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 heifers were housed in semi-open sheds.

Experimental design:

The experimental heifers in all groups had mature CL sand were injected intramuscularly with 2.5 ml/animal of PGF2α analogue (Estrumate, Essex Animal Health Fresoythe Sedelsberger Strasse 2-4. 26169 Friesoythe, Germany) containing 0.625 µg/ml Cloprostenol sodium to synchronize the estrous cycle. At the end of treatment, estrus was detected for 72 h at every morning and evening by close observation for external signs in presence of a teaser buffalo bull all the times of observation.

Heifers in heat were naturally inseminated by fertile buffalo bull and allotted randomly to four experimental groups (5 animals in each). Heifers in the 1st group (G1) were injected with 2.5 ml saline at the time of mating and were considered as control group. However, heifers in the 2nd, 3rd and 4th groups were injected intramuscularly 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 (G2), 10 (G3) and 12 (G4) from estrus onset, respectively.Each ml Receptal contained 4µg Buserelin acetate.

Blood samples:

Blood samples were collected by jugular venipuncture from all animals of each group on days0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 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 and transferred into sterilized serum vials. All samples were stored at -20 °C to for determination of progesterone (P_4) concentration in blood serum.

Hormone assay*:*

Direct Radioimmunoassay technique (RIA) was performed for determination of serum P_4 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 in non-returned animal by rectal palpation on day 45-50 post-mating. Then conception rate was calculated.

Statistical analysis:

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

RESULTS AND DISCUSSION

Conception rate:

Data in table (1) showed that the conception rate (CR) was significantly ($P<0.05$) the highest in G3 and G4 (80%), moderate in G2 (60%) and the lowest in G1 (40%).This means that GnRH injections on day 10 or 12 of estrus were more effective on improving CR as compared to that injected on day 0, which significantly ($P<0.05$) increased CR as compared to that of control group (G1). Such results indicated that GnRH administration on 0, 10 and 12 days of estrus had significant ($P<0.05$) impact on CR of buffalo heifers.

In accordance with the present results, Karimi *et al*. (2007) showed 10% improvement in CR (70 *vs.* 80%) when GnRH was administered to dairy heifers on day of estrus. Attoo *et al*. (2013) found that the GnRH agonist administration improved CR in buffaloes when administered on day 13 post-breeding (80%) compared to day 0 (70%) or day 11 (70%). Mandal *et al*. (2009) reported an improvement in CR (75.0 *vs.* 87.5%) of buffaloes treated with GnRH on day of estrus as compared to control. However, in cattle, Iftikhar *et al*. (2009) also observed improvement in CR (37.5 *vs.* 68.75%) when GnRH was injected at the time of insemination. Rangnekar *et al*. (2002) reported CR of 70% in repeat breeder Holstein-Friesian cows.

Table 1. Conception rate (%) of buffalo heifers in the experimental groups

Experimental group	Number of animals	Animals		Conception rate (%)
		Pregnant	Non pregnant	
G1 (Control)	5	2	3	40[c]
G2 (day 0)	5	3	2	60[b]
G3 (day 10)	5	4	1	80[a]
G4 (day 12)	5	4	1	80[a]

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

Failure of ovulation might be prevented and CR might increase by GnRH administered at AI. The improvement in CR of buffalo heifers 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), 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).On the other hand, Perry and Perry (2009) found that the GnRH administration failed to exhibit a positive impact on pregnancy rate. 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:

Results presented in table (2) indicated significant (P<0.05) differences in serum P_4 concentration among the experimental groups on most sampling days. Serum P_4 concentration was significantly (P<0.05) the highest in G4 on most sampling days. Results also showed insignificant differences in serum P_4 on day of estrus, being ≤0.5 ng/ml in all groups, and indicating incidence of estrus and ovulation as well as complete regression of all CLs presented at the beginning of treatment in all heifers used in this study. It is well known that all animals used in this study were treated with PGF2α to induce estrus/ovulation of treated heifers. Many authors reported similar findings on Egyptian buffalo cows (El-Moghazy *et al*., 2006) and buffalo heifers (Aboul-Ela *et al*., 2006).

It is of interest to note that P4 concentration was the highest in G2 as compared to other groups, showing response of animals in these groups to GnRH injection on day of estrus. However, the increase in P4 concentration in G3 compared with G1 (control) was observed staring on day 14 up to day 24. Such finding revealed lower response of animals in this group to GnRH following GnRH injection on day 10. However, heifers in G4 showed the highest response to GnRH injection on day 12, whereas P4 concentration tended to increase on day of GnRH injection.

The present results are in agreement with Attoo *et al*. (2013), who showed significant differences (P<0.05) 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) 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)or in buffaloes (Mandal *et al*., 2009).

Table 2. Progesterone (P_4) concentration (ng/ml) in blood serum of buffalo heifers in the experimental groups

Day post-mating	G1 (Control)	GnRH treatment		
		G2 (day 0)	G3 (day 10)	G4 (day 12)
0	0.481±0.04	0.453±0.0	0.422±0.03	0.410±0.03
2	0.790±0.10[c]	1.59±0.14[a]	1.194±0.10[b]	0.692±0.05[c]
4	1.20±0.05[b]	1.82±0.23[ab]	1.330±0.08[b]	2.024±0.26[a]
6	2.98±0.09	2.91±0.19	2.782±0.23	3.502±0.40
8	3.30±0.37[b]	3.77±0.19[ab]	3.752±0.23[ab]	4.590±0.42[a]
10	3.40±0.17	3.91±0.16	3.724±0.24	3.766±0.28
12	3.27±0.32[b]	3.79±0.28[ab]	4.110±0.05[ab]	4.636±0.33[a]
14	4.24±0.22[b]	4.32±0.20[b]	4.974±0.22[a]	5.436±0.17[a]
16	4.39±0.27[b]	4.95±0.29[b]	5.036±0.33[ab]	6.006±0.38[a]
18	3.92±0.63[b]	4.64±0.22[ab]	5.564±0.38[a]	5.582±0.23[a]
20	2.49±0.93	4.29±0.92	5.102±0.67	5.048±0.65
22	2.70±0.29[c]	3.62±0.21[bc]	4.294±0.25[ab]	5.122±0.32[a]
24	2.18±0.90[c]	3.02±1.05[bc]	4.308±0.96[ab]	4.644±1.11[a]

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

In addition, the present results indicated marked group differences in P4 concentration on days 22 and 24, but P4 concentration was always ≥2 ng/ml in all groups. This may indicate incidence of pregnancy with different trends among groups. It is worth noting that P4 level was in accordance with CR (number of pregnant animals) in each group (Table 1), being significantly (P<0.05) the highest in G4 and G3, followed by G2 and the lowest in G1 (Table 2).

Concerning the overall concentration of P_4 in pregnant and non-pregnant heifers in each group (Fig. 1), the statistical analysis revealed that P4 level was significantly (P<0.05) higher in pregnant than in non-pregnant heifers. Cows were regarded to be pregnant when the P_4 concentration in serum was ≥1.6 ng/ml on day 24 post-mating (Yildiz *et al*., 2009). The later author found that pregnancy rate in cows treated with GnRH was 77.7% compared with 50% in control group. Also, some authors (Tefera *et al*., 2001; Howard *et al*., 2006) showed that injection of GnRH on days 11 to 14 after AI in lactating cows increased serum P_4 level**.**

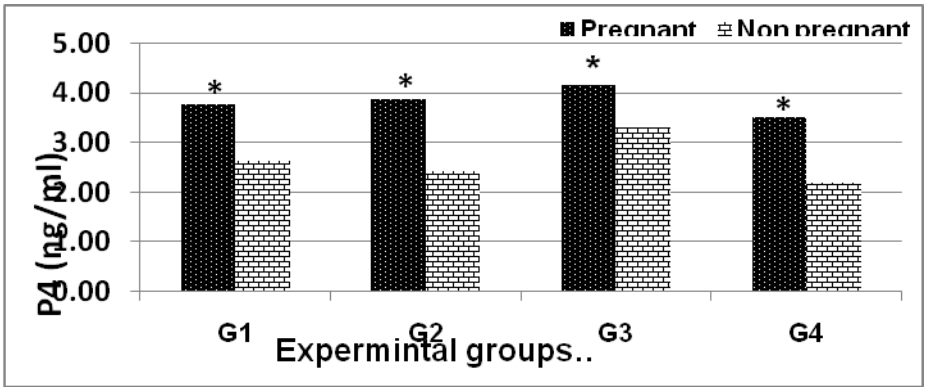

Fig. 1. Progesterone (P4) concentration (ng/ml) in pregnant and non-pregnant buffalo heifers of the experimental groups

As a result of GnRH administration at estrus induces, Aboul-Ela *et al*. (1985) suggested release of both LH and FSH in buffaloes which causes 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. Also, Campanile *et al*. (2007) found that the 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

CONCLUSION

Based on the foregoing results, the current study indicated that GnRH analogue (Receptal) administration at a level of 2.5 ml/animal on day 12 post-mating improves pregnancy rate of repeat breeder buffalo heifers in comparison with day 0 or day 10 in term of positive effects on enhancing luteal function by elevating progesterone level. Further studies are required to evaluate different days during different days post-mating of repeat breeder heifers.

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تأثير المعاملة بـ GnRH أثناء أوقات مختلفة بعد التلقيح على معدل الحمل فى عجلات الجاموس

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تهدف هذه الدراسة الى تقييم كفاءة الحقن بالهرمون المنشط لافراز الهرمونات الجونادوتروفيه (GnRH) فى يوم التلقيح (صفر) واليوم ١٠ واليوم ١٢ بعد التلقيح على معدل الحمل فى عجلات الجاموس متكررة التلقيح. استخدم فى هذه الدراسة عدد (٢٠) من عجلات الجاموس المصرى متكررة التلقيح (لم تخصب بعد ثلاث تلقيحات على الاقل) عمرها من ٢-٤ سنوات ووزن حى من ٣٥٠-٤٠٠ كجم تم تقسيمهاعشوائيا الى ٤ مجموعات كل مجموعة تحتوى على ٥ حيوانات. تم حقن جميع العجلات والتى كانت تحتوى على أجسام صفراء ناضجة بـ٢.٥مل بروستاجلاندين (استروميت) لكل عجلة. تم تلقيح العجلات التى اظهرت شياع (بعد الحقن بالبروستاجلاندين) خلال ٧٢ ساعة فى كل المجاميع طبيعيا بفحل ناضج جنسيا وفى يوم الشياع تم حقن العجلات بـ٢.٥ مل محلول ملح فسيولوجى لكل عجلة (المجموعة الاولى- كنترول)، وتم حقن المجموعة الثانية بـ ٢.٥ مل ريسبتال لكل عجلة فى يوم الشياع والتلقيح (اليوم صفر) والمجموعة الثالثة والرابعة تم حقنها بـ ٢.٥ مل ريسبتال لكل عجلة فى اليوم ١٠ و١٢ بعد التلقيح . تم جمع عينات الدم لتقدير تركيز البروجسترون فى سيرم الدم . وتم تشخيص الحمل فى كل حيوان بعد ٢٥ يوم من التلقيح باستخدام جهاز الموجات فوق الصوتية. وقد اوضحت النتائج ما يلى:

١- كان معدل الحمل أعلى معنويا ($P<0.05$) فى المجموعة الثالثة والرابعة (٨٠% لكل منها) و متوسطا فى المجموعة الثانية (٦٠%) ومنخفضا فى المجموعة الاولى (٤٠%).

٢- أرتفع تركيز البروجسترون فى سيرم الدم معنويا ($P<0.05$) فى المجموعة الرابعة فى معظم الايام التى تم اخذ العينات فيها مقارنة بالمجاميع الاخرى.

٣- أظهرت عجلات المجموعة الرابعة أعلى أستجابة للحقن بالهرمون المنشط لافراز الهرمونات الجونادوتروفيه فى اليوم ١٢ بعد التلقيح حيث زاد تركيز البروجسترون من يوم الحقن بالهرمون المنشط لافراز الهرمونات الجونادوتروفيه فى هذه المجموعة .

٤- لوحظ وجود اختلافات بين المجاميع فى تركيز البروجسترون فى اليوم ٢٢ و٢٤ ولكن كان تركيز البروجسترون أعلى معنويا ($P<0.05$) فى عجلات الجاموس العشارعن غير العشار داخل كل مجموعة.

نستخلص من هذه الدراسة ان المعاملة بالهرمون المنشط لافراز الهرمونات الجونادوتروفيه (٢.٥مل/عجلة ريسبتال) فى اليوم ١٢من الشياع/التلقيح أدى الى أعلى تأثير ايجابى على معدل الحمل فى عجلات الجاموس المصرى متكررة التلقيح بالمقارنة مع تلك التى تم حقنها فى اليوم صفر واليوم العاشر من التلقيح .