ESTROGEN AND PROGESTERONE PROFILES DURING SUPEROVULATION IN BUFFALO HEIFERS WITH HIGH AND LOW OVULATORY RESPONSE

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ABSTRACT

Fifteen buffalo heifers of similar age and weight investigated during mid-luteal phase. Ten superovulated with pFSH (65 NIH units, Super-Ov, divided into 6 equal doses, injected for 3 consecutive days) and 25 mg Lutalyse given with fifth injection. LH (2000 USP unit) injected 12 hour (h) from treatment. Rest 5 heifers kept as control. Blood samples taken before treatment (0h) then after 12, 24, 48, 72, 96, 120 and 144 h. Sera collected and kept at −20 C° for estrogen (e) and progesterone (p) determinations using Enzyme Immunometric Analyzer. Superovulated heifers slaughtered after last blood samples to count corpora lutea (CL) and unovulated follicles (UF). Oviducts and uterus flushed to recover embryos. Results classified into high response group (HRG) > 2 CL (3.8 ± 0.49) and low response group (LRG) < 3 CL (1.2 ± 0.2). Nine embryos recovered from HRG where significant increase in e noticed at 72 h followed by marked decrease. The increased e in LRG continued till last sample with significant difference (P < 0.05). The total number of UF was significantly lower (P<0.05) in HRG. The overall p levels (ng/ml) were significantly lower (P<0.01) in HRG than LRG and control. Lutalyse induced significant reduction in p after 2 days from injection in HRG only. Wide variations in p levels (ng/ml) were noticed in buffalo heifers at the start of treatment, during mid luteal phase and low concentrations reported with HRG (0.87±0.33). Therefore, low levels of p at the beginning of superovulation could indicate successful ovulatory response.

INTRODUCTION

Superovulation is a topic of great interest in animal reproduction since many of the literature showing a contrasting superovulatory response.

Uoc *et al.* (1997) showed that the supplementation of estrogen and human chorionic gonadotropin treatment significantly improved the level of ovarian stimulation in Swamp buffalo. The same authors declared that the level of ovarian stimulation in buffalo is still unpredictable and low. Osman *et al.* (2001) reported that 2000 USP unit chorionic gonadotropin injected at the 5th day of treatment improved the rate of ovulation in buffalo cows rather than in buffalo heifers when superovulated with pFSH administered for 3 consecutive days. Donaldson (1985) mentioned that neither ovarian size, number of corpora lutea nor blood progesterone levels were correlated with embryo production.

The suppressive effects of dominant follicle and as well the endocrine disorders are among the factors affecting superovulatory response in cattle (Kafi and McGowan, 1997). Moreover, Madan *et al.* (1996) concluded that UF at superovulation contribute high quantity of estrogen altering the uterine milieu. Beg *et al.* (1997) declared that no significant difference was observed in ovulatory response of buffalo superovulated in mid or late luteal phase (days 8-12 or 13-15 of the estrous cycle). Mervat (2007) attributed the presence of follicles with variable sizes during all phases of estrous cycles to the continuous process of folliculogenesis in buffaloes.

Roberge *et al.* (1995) cited that in superovulated Holstein heifers, 30 hour after prostaglandin treatment, serum estradiol concentration and LH pulse frequency were positively correlated with the number of CL, total number of embryos and transferable number of embryos recovered. In superovulated cows, the suppression of LH and FSH secretion was caused by the negative effect of higher concentrations of estradiol and progesterone in the hypothalamic-hypophysial axis (Roberge *et al.*, 1995 and Pant, 2000).

Positive correlation between superovulation and plasma progesterone at the time of gonadotropin

treatment has been demonstrated in buffalo (Madan *et al.*, 1993) and cattle (Gradela *et al.,* 1996 and Beg *et al.,* 1997). However, Greve *et al.* (1983) and Lindsell *et al.* (1986) claimed to the contrary in cattle. Misra *et al.* (2000) found that concentration of progesterone on the day of superovulatory treatment and estrogen concentration on day 2 of superovulatory cycle were positively correlated with ovulation rate. McEvay *et al.* (1996) cited that neither feed restriction nor supplementary progesterone prior to ovulation influence the proportionate yields of viable embryos at day 7 after estrus in Simmental heifers.

Ismail *et al.* (1992) and Misra *et al.* (2000) indicated that measurement of circulating progesterone at the start of gonadotropin injection may be a useful index of superovulatory response and embryo recovery in multiparous buffalo cows and high levels of progesterone gave better results.

The present work aimed to throw some lights on the event of changes occurring in the blood serum estrogen and progesterone during superovulation in buffalo heifers with contrasting high and low superovulatory response as a trial to predict the success of ovarian stimulus at the start of superovulation.

MATERIALS and METHODS

From a local governmental buffalo farm, 15 heifers at mid luteal phase (day 10-12 of the estrous cycle) were selected. These animals were healthy and raised under the same environmental condition of management and feeding. They aged 21-25 month and weighed 325-385 kg. Ten heifers of them were superovulated with pFSH (Total 65 NIH unit, Super-Ov, divided into 6 equal doses, injected for 3 consecutive days) and Lutalyse (25 mg given with the fifth injection). LH doses (2000 USP unit) were injected 12 hour from pFSH treatment. The amount of pFSH which used in the present investigation was applied according to the recommendation of Osman *et al.* (2001) for buffalo heifers. The rest 5 heifers were injected with saline and served as control.

Blood samples were taken from the jugular vein just before treatment (0 hour) then after 12, 24, 48, 72, 96, 120 and 144 hour. After clotting and centrifugation, sera were collected and kept at -20 Cº for e and p determinations using Immulite Enzyme Immunometric Analyzer (Randolf, N.J., USA).

All heifers were slaughtered, just after the last blood sampling, and their genitalia were taken to the laboratory for further investigations. The numbers of newly formed CL and UF were taken. Moreover, all UF were measured to the nearest 1/10 cm. using a metal caliber. Flushing of the oviducts and uterine horns were made with saline to count the recovered embryos using a stereo microscope.

The obtained data were statistically analyzed using the student t-test and simple correlations between two factors according to SAS computer system version 3.2 and Snedecor (1980).

RESULTS

The obtained results are presented in tables 1-3. The number of new CL and UF (<0.8cm) showed significant difference (P<0.05) between HRG and LRG (Table 1). Moreover, embryos were recovered only from HRG. Although these animals were selected during mid luteal phase and were nearly similar in age and live body weight, at the start of superovulation, varying degrees of ovarian response were observed at the end of the treatment.

Estrogen profiles (Table 2) showed insignificant changes between groups, although the overall mean was higher in the superovulated heifers than control (28.26±4.1 pg/ml versus 25.62±3.6 pg/ml respectively). The higher levels reported for estrogen in HRG than LRG and control at 24, 48 and 72 h from FSH injection were accompanied with higher incidence of ovulation and CL formation. Moreover, within HRG, there was a significant increase (P<0.05) in estrogen level at the time of LH injection after 72 hour (onset of estrus) from treatment followed by marked decrease till the end of trial at 144 hour. This decrease coincides with the observed low number of small UF (< 0.8 cm, Table 1). The reverse was true within the LRG in which significant increase in e was observed between 96 and 144 hour of the treatment when a significant increase in the number of small UF was noticed without any recovered embryos after flushing the uterine horns and oviducts.

During the last 2 days of the study, at 120 and 144 hour, significant correlation was present between the number of large UF (>0.7 cm) and e levels in treated groups (r=0.65, P<0.05). One day after LH injection, e levels decreased in all treated animals. The total number of UF in LRG was 37 versus 23 in HRG.

Out of table 3, significant (p<0.01) low concentrations of p were reported with HRG (0.87±0.33) in comparison with the LRG (3.67± 1.25) and control (1.57± 0.23) at the first day of injection. These low levels of p in the HRG remained lower till the last day of treatment. Moreover, the overall p levels were significantly lower (P<0.05) in HRG than the other two groups (0.86± 0.15 versus 2.97± 0.38 LRG and 2.3± 0.21 control). It is evident that heifers with low levels of p at the start of superovulation responded much better for superovulation than the others.

Lutalyse induced significant reduction in p levels after 2 days in HRG only (between 12 and 96 hour, P<0.05). Significant correlation between p and CL was present among treated groups, during the last 2 days of treatment (r=0.85, P< 0.01). The continued decrease in p till the end of study, after Lutalyse injection, in the LRG, reflected the decreased number of successful ovulation with a consequent low number of new CL.

The average increase in p during the last 2 days of treatment in relation to the previous days were 2.27 fold in HRG versus 0.54 fold increase in LRG and 1.27 in control group. With regard to e such pattern of increase showed contrary results: 0.75 fold increase in HRG versus 1.21 fold in LRG and 0.87 fold in control group. Marked increase in p accompanied with marked decrease in e during the last 2 days of treatment (Day of embryo collection) could reflect high ovarian response to superovulation and embryos yield.

Table 1: Distribution of New Corpora Lutea, Unovulated Follicles and Recovered Embryos in High and Low Response Superovulated Buffalo Heifers.

Superovulated Buffalo Heifers	New Corpora Lutea	Unovulated Follicles (> 0.7 cm)	Unovulated Follicles (< 0.8 cm)	Recovered Embryos
High Response Group	3.8 ± 0.49 *	1.6 ± 0.51	3.0 ± 0.77 *	1.8 ± 0.76
Low Response Group	1.2 ± 0.20	1.0 ± 0.32	6.4 ± 0.68	00.00

N= 5
Mean ± SEM
* Significant (P < 0.05)within column

Table 2: Estrogen Profile (pg / ml) During Superovulation in Buffalo Heifers with Variable Ovarian Response

Regime of Superovulation	Control Group (n = 3)	High Ovarian Response (n = 5)	Low Ovarian Response (n = 5)
Before Treatment (1)	24.67 ± 1.04	26.42 ± 2.59*	31.96 ±5.45
After Treatment (hour) :			
0 (2)			
12	27.33 ± 0.96	29.82 ± 2.81	29.32 ±4.33
24	25.65 ± 1.64	31.72 ± 4.15	24.26 ±2.26
48 (3)	29.01 ± 0.67	31.98 ± 5.22	26.92 ±2.50
72 (4) (Estrus)	30.0 ± 1.78	36.24 ± 7.38 *	26.34 ±2.80
96 (Estrus)	22.3 ± 1.92	24.2 ± 2.72	22.52 ±3.50 *
120	27.8 ± 1.86	22.28 ± 4.71	32.50 ±7.54
144 (Day of Slaughter)	18.20 ± 0.98	23.02 ± 1.83	32.82 ± 8.30 *

Total Mean ± St. Er.	25.62 ± 1.28	28.21 ± 1.63	28.33 ± 1.29

Mean ± SEM
(1) At Mid-luteal phase
(1) pFSH Injection
(2) Lutalyse Injection
(3) LH Injection
High Response Group : Ovaries with > 2 CL
Low Response Group : Ovaries with < 2 CL
* Significant Difference within Group (P< 0.05)

Table 3: Progesterone Profile (ng / ml) During Superovulation in Buffalo Heifers with Variable Ovarian Response.

Regime of Superovulation	Control Group		High Response Group Ovaries with > 2CL		Low Response Group Ovaries with < 2 CL	
Before Treatment (1)	1.57	± 0.23 *	0.87	± 0.33 *	3.67	± 1.25 *
After Treatment (hour):						
0 (2)						
12	1.47	± 0.19	1.04	± 0.21 *	4.86	± 1.33 *
24	1.63	± 0.19	0.88	± 0.34	4.34	± 1.28
48 (3)	2.73	± 0.17	0.32	± 0.12 *	2.16	± 0.54 *
72 (4) (Estrus)	2.80	± 0.22	0.57	± 0.23 *	2.46	± 0.64 *
96 (Estrus)	2.77	± 0.15	0.25	± 0.03	2.40	± 0.89
120	2.93	± 0.26	1.46	± 0.59	2.0	± 1.63
144 (Day of slaughter)	2.58	± 0.25	1.52	± 0.83	1.86	± 0.71

Total Mean	2.31	± 0.21	0.86	± 0.15	2.97	± 0.38

(N = 5) Mean ± SEM   (1) At Mid-luteal phase   (2) pFSH Injection
 (3) Lutalyse Injection   (4) LH Injection
* Significant Difference between groups (P < 0.05)
* Significant difference within HRG between 12 and 96 hour (P < 0.05)

DISCUSSION

Although the investigated buffalo heifers were selected during mid luteal phase, at the start of superovulation, varying degrees of ovarian response was observed at the end of treatment. This means great individual variations in reaction of the ovaries to superovulation in buffalo heifers even with nearly similar ages, body weight and other managerial conditions of breeding. Our attention to classify heifers into high and low response groups based on the wide variations of the obtained results. Kafi and McGowan (1997) and Mohammed (2000) cited about the variability of the superovulatory response in cattle and buffalo to be a primer difficulty in successful embryo transfer program.

The obtained results with regard to ovulations and number of recovered embryos agree with those published by El Sherry (2003) in superovulated buffalo heifers, but much less than those reported in dairy cattle by Feuntes and De La Fuente (2000) who recovered 12.8 ± 7.5 embryos from superovulated Holstein heifers. Drost (2007) attributed the much lower rate of success in embryo transfer techniques in water buffaloes than those in cattle to their inherent lower fertility and poor superovulatory response. Moreover, Presicce et al. (2004) concluded that many significant morphological and endocrine differences were present between nuliparus and mixed parity

Mediterranean Italian buffaloes following estrus synchronization.

Estrogen profiles showed insignificant differences between groups, although the overall mean was higher in the superovulated heifers than control (28.26 ± 4.1pg/ml, versus 25.62 ± 3.6pg/ml, control). These findings coincide with the results of Sunilchandra et al. (2001) who reported that peak estradiol concentration was significantly greater in buffalo treated with follicle stimulating hormone than that of control. Within HRG, there was a significant increase (P<0.05) in e level at the time of LH injection after 72 h from treatment (during estrus) followed by marked decrease till the end of trial at 144 h (Day 6). This decrease could be attributed to the observed low number of small UF (less than 0.8 cm, Table 1) since the other larger follicles ovulated. Roy and Parkash (2008) found that estrus behavior was positively correlated with peak concentration of estradiol-17 β and total e, during the peri-estrus period in buffalo heifers with induced ovulation. The higher levels of e in HRG than LRG and control at 24, 48 and 72 h from FSH injection were accompanied with improved rate of ovulations. Thus, high levels of e during this period may be essential for successful superovulation.

In LRG the significant increase in e was observed between 96 and 144 hour of treatment when a significant increase in the number of small UF was

noticed without any recovered embryos. The circulating estrogen in the same group reached a peak level at day of slaughter. Within the scope of these findings, Roy and Parkash (2008) found that estrus behavior was positively correlated with peak concentration of estradiol-17β and total e, during the peri-estrus period in buffalo heifers with induced ovulation.

It might be possible that estimation of e at the day of embryo collection (Day 6) in buffalo heifer can be used as a criterion to predict the success of superovulation and yield embryos since high levels of estrogen at this day were accompanied with poor ovulation rate.

In the present work, peak estrogen concentration (32.82 ± 8.3 pg/ml) was noticed at day of slaughter (144 hour from treatment) in LRG where the highest number (6.4 ± 0.68) of small UF (less than 0,8 cm) was recorded (table 1). This may lead us to conclude that UF of variable size could contribute more estrogen to the animal during superovulation. The present results are in agreement with Madan *et al.* (1996). However, Ginther *et al.* (2000) reported that in cattle the largest follicles release estradiol into the blood at the beginning of follicle deviation during the mid-luteal phase. On the other hand, Gibbons *et al.* (1999) reported that 5-mm follicles were not associated with increased estradiol levels in cattle. The day of follicle deviation was reported by El Sherry (2003) to be the 10th day of estrous cycle in buffalo heifers. Thus, the dominant follicle may play a role in the high level of e at this period in which the present trial was started.

In HRG, the superovulated heifers reacted more satisfactory with the FSH gonadotropin treatment since the levels of e were higher, during injections, than other groups. Such high levels of e may be required for the full growth of the developed follicles and as well for their ovulations and CL formations. In LRG where the e levels were lower during the first 2 days of treatment, the total number of UF was higher (37 versus 23 in HRG). Fuentes and De La Fuente (2000) used exogenous estradiol plus p to improve embryo production in superovulated Holstein dairy herds.

The significant differences in p levels between groups at the start of treatment indicate wide individual variations in the functional activity of CL during mid luteal phase in buffalo heifers. Moreover, animals with low levels of p at the beginning of superovulation responded much better than others. It is noticed here that week response of the ovaries for superovulation is expected to occur when buffalo heifer has a high concentration of p at the beginning of the regime. Misra *et al.* (2000) reported contrasting results in buffalo cows.

The overall concentrations of p (ng/ml) were significantly lower in HRG than control (0.86±0.15 versus 2.97±0.38). Within the scope of this area, El Sherry *et al.* (2011) cited that the superovulated CL has a smaller size, shorter lifespan and less P production in comparison with the cyclic CL.

The significant reduction in progesterone levels, after 2 days from Lutalyse injection in HRG only (between 12 and 96 hour, P<0.05) concomitantly run with the initial low levels of this hormone at the start of treatment. This may indicate rapid autolysis of the weekly active CL, which secretes low p during mid luteal phase in buffalo heifers, in response to Lutalyse treatment. Such marked autolysis of the CL may play a role in the success of superovulation in HRG. In agreement with these results, El Sherry *et al.* (2010) reported that low p level during FSH treatment enhanced multiple follicular growth and estradiol secretion. They added that absence of CL may play a critical role in the superovulation response by controlling the number of growing follicles.

Significant correlation between progesterone and corpora lutea was present among treated groups, during the last 2 days of treatment. In HRG progesterone showed significant increase (r=0.85, P< 0.01) after 72h (estrus period) in association with the newly formed CL. Although positive correlation between superovulatory response and plasma progesterone at the time of gonadotropin treatment has been demonstrated in buffalo by Madan *et al.* (1993), Ullah *et al.* (1992) claims to the contrary in Nili-Ravi buffalo.

During the last 2 days of treatment the mean p levels were nearly double that before treatment in HRG and the reverse was true in LRG. These findings coincide with Papa *et al.* (2007) who reported that in superovulatory buffalo the CL is able to increase the translation rate of the vascular endothelial growth factor of the luteal cells to produce progesterone.

On the other hand, the continued decrease in progesterone till the end of study, after Lutalyse injection, in LRG, reflected the decreased rates of successful ovulation and CL formation.

It is evident from table 3, that the lowest levels of p were noticed before and during estrus in HRG only (0.32 ng/ml average mean values). After that the value increased up to 1.49 ng/ml i.e. 2.27 fold increase. With regard to e (table 2) 1.21 fold increase was noticed in the low response group during the last 2 days of treatment, after the end of estrus. Both of these findings correlated well with the success or failure of superovulation protocol in buffalo heifers respectively. Such observation reflects the role of e and p profiles in the success of superovulation. In harmony with these results, Roy and Parkash (2008) reported that plasma p concentration in Murrah

buffalo heifers was basal (0.2 ± 0.001 ng/ml) during the peri-estrus period and increased to a peak level on day 13.0 ± 2.0 of the cycle. It seems possible that the physiological status of the animal at the start of superovulation, especially with regard to ratio between e and p concentrations, played a major role in the response of the ovaries to the treatment and low p accompanied with high e give the best results.

In high response group, the superovulated heifers reacted more satisfactory with the FSH gonadotropin treatment since the levels of e were higher, during injections, than other groups. Such high levels of e may be required for the full growth of the developed follicles and as well for their ovulations and CL formations.

As a major conclusion from the present work, week response of the ovaries for superovulation is expected to occur when the animal has a high concentration of p at the beginning of the regime. Moreover, estimation of e at the day of embryo collection (Day 6 in buffalo heifer) can be used as a criterion to predict the success of superovulation and high levels pointed to low ovarian response.

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صورة الاستروجين والبروجستيرون فى شابات الجاموس خلال التبويض الفائق مع الاستجابة العالية والمنخفضة

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تم فى هذا البحث استخدام 15 عزبة جاموس من مزرعة الحواتكه بأسيوط تراوحت أعمارهم من 21-25 شهراً وأوزانهم من 325-385 كيلو جرام. عشرة من هذه الحيوانات تم حقنهم باستخدام الهرمون الحاث للحريبات 65 وحده NIH قسمت الى 6 جرعات متساوية على ثلاث أيام متتاليه (حقنه كل 12 ساعة) وباق الحيوانات وعددها 5 تركت كمجموعة ضابطة. تم حقن حيوانات التجربة بالهرمون المنبه للجسم الأصفر LH لتنشيط التبويض بجرعة ألفين وحده usp بعد 12ساعة من بداية الحقن. كافة هذه الحيوانات تم حقنها أثناء الفترة الوسطى من وجود الجسم الأصفر. أخذت عينات دم قبل بداية الحقن مباشرة ثم بعد 12، 24، 48، 72، 96، 120، 144 ساعة من الحقنة الأولى. تم فصل السيرم وحفظ عند سالب 20م°حتى تقدير الاستروجين والبروجستيرون باستخدام التحلل الانزيمى المناعي. بعد نهاية التجربة ذبحت الحيوانات وتم فحص المبايض وعد الحريبات والأجسام الصفراء الجديدة. وتم عمل غسول لقناة فالوب والرحم لعد البيوضات المخصبة (الأجنة). من نتائج هذه التجربة وفى المجموعة الضابطة كان المعدل الكلى للاستروجين 1.04 ± 24.6 بيكوجرام /مللى قبل الحقن ثم أرتفع إلى 1.78± 30.0عند 72ساعة وانخفض إلى 0.89 ± 18.2عند 144ساعة أما معدل البروجستيرون (نانوجرام / مللى) فكان 0.23 ± 1.57 قبل الحقن ثم ارتفع الى 0.26±2.9 عند 120 ساعة واصبح 2.58±0.25 عند 144 ساعة – تم تقسيم جاموس التجربة إلى مجموعتين: الأولى: ذات الاستجابة العالية (بها أكثر 2 جسم أصفر حديث التكوين بمعدل 0.49 ±3.8) والثانية: ضعيفة الاستجابة (بها اقل من 3 جسم أصفر حديث التكوين بمعدل 0.2±1.2) والفرق بين تلك المجموعتين له قيمة معنويه عالية - تبين وجود ارتفاع معنوي في الاستروجين عند 72 ساعة في المجموعة الأولى حيث انخفض بشده بعد ذلك أما المجموعة الثانية فقد استمر معدل الارتفاع حتى نهاية التجربة بفروق معنوية. كان هناك علاقة وطيده وايجابية بين مستوى الاستروجين وعدد الحريبات خلال اليومين الآخرين من التجربة. تبين أن عدد الحريبات التى لم يحدث لها تبويض كان قليلا في المجموعة الأولى عنة في المجموعة الثانية (23 حريبة مقابل 37). بعد يوم واحد من حقن LH حدث انخفاض في مستوى الاستروجين لكل الحيوانات. المعدل الكلى لمستوى البروجستيرون كان منخفضاً في المجموعة الأولى باقي المجموعات (0.15 ± 0.86 مقابل 0.21 ± 2.3 في المجموعة الضابطة و 0.38 ± 2.97 في المجموعة الثانية ذات الاستجابة الضعيفة). عقار الـ Lutalyse أحدث انخفاض معنويا شديدا في البروجستيرون بعد يومين من الحقن في المجموعة الأولى فقط وكانت هناك علاقة وطيده ذات قيمة معنوية عالية بين مستوى البروجستيرون وعدد أجسام الاصفر حديثة التكوين في كلتا المجموعتين خلال اليومين الآخرين من التجربة. يستنتج من نتائج هذا البحث أن انخفاض مستوى البروجستيرون إلى الاستروجين في مصل الجاموس قبل بداية تجربة التبويض الفائق له دور في توقع ارتفاع نسبة نجاح استجابة المبايض للتبويض.