

FOLLICULAR DYNAMICS, OVULATION AND REPRODUCTIVE HORMONES FOLLOWING GnRH PRE-TREATMENT IN POST PARTUM SUCKLED DAIRY BUFFALO- COWS

BR. ABDEL-HALIM* and NERMEEN A. HELMY**

*Department of Theriogenology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt, Tel/ Fax:0020822327982.

**Department of Physiology, Faculty of Veterinary Medicine, Beni- Suef University, Beni-Suef, Egypt.
Email: drbakarwa@yahoo.com

ABSTRACT

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The objective of this study was to investigate the role of administration of gonadotropin-releasing hormone (GnRH) at day 12-14 postpartum on ovarian dynamics and conception rates in buffalo- cows. The study was conducted on twenty four, apparently healthy, lactating buffalo-cows in their second to fifth parity. The animals were divided into two groups; group I (n=10) considered as control group that did not receive any treatment and group II (n=14) that received 100 µg GnRH (Receptal®) for each animal during the period from 12-14 days postpartum. Ovarian structures were monitored daily, from day 6 postpartum until detection of the third postpartum ovulation, using a real- time ultrasound. Blood samples were collected via the coccygeal vein from day 6 post treatment and every 3 days until detecting the third postpartum ovulation to estimate serum progesterone, serum luteinizing hormone, and Follicle stimulating hormone profiles. All animals ovulated before day 45 postpartum in GnRH treated group (14/14,100%); whereas, none of the cows in control group ovulated within this period of time. Interval from calving to the first ovulation and from calving to the second and the third ovulations in GnRH treated group was significantly shorter (23.6±1.4, 45.2±2.3 and 67.8±1.9 days respectively) than in control group (65.1±5.8, 87.6±6.4 and 119.2±6.3 days respectively). The mean serum progesterone concentrations were significantly higher in treated group in the first and the second cycle (3.6 ± 0.2 and 4.1 ± 0.1 ng/ml) than in the control group (1.8 ± 0.3 and 3.1 ± 0.6 ng/ml respectively). The pattern of LH release was similar in both treated and control groups, while the peak LH was significantly higher in treated group (21.4 ± 3.8 ng/ml) than the control group (12.8 ± 1.9 ng/ml). The actual intervals from calving to first behavioral estrus and the actual interval from calving to conception (days open) were significantly shorter in treated group (42.6 ± 1.8 days, 86.9 ± 1.6 days respectively) than the control group (65.2 ± 3.4, 100.4 ± 2.6 days respectively). The conception rate was higher in treated group (77.4 ± 1.9) compared to control group (55.8 ± 2.6). From the current study we concluded that, GnRH treatment on day 12-14 postpartum stimulate early ovulation in buffalo- cows, thus stimulate the postpartum behavioral estrus, shorten number of days open, decrease number of services per conception and improve the conception rate.

Keywords: GnRH; ovarian dynamics; hormonal profile; conception rate; buffalo-cows.

INTRODUCTION

Buffalo-cows (*Bubalus bubalis*) are of high economic importance in various developing countries but reproductive performance is poor. The incidence of anoestrus in buffaloes ranges from 20 to 80 % depending on seasonal factors (Nanda *et al.*, 2003).

Most buffaloes cease ovarian cyclicality during hot summers probably due to the combined effects of nutrition, environment and management (Das and Khan, 2010). The reproductive process in buffalo-cows is very slow characterized by delayed onset of puberty, long postpartum anoestrus and an extended inter-calving interval (Nanda *et al.*, 2003). Suckled buffaloes have significantly delayed onset of

postpartum ovarian activity (Arya and Madan, 2001). The poor reproductive performance, especially in postpartum buffaloes, could be attributed to suckling, which is commonly practiced to induce milk let-down in smallholder dairy-farming systems (Sastri *et al.*, 1994). Suckling, an exteroceptive stimulus, suppresses episodic pituitary gonadotropin leading to anoestrus and / or anovulation in several female mammals, including sheep, rat, monkey and humans (McNeilly, 1988). However, the effects of suckling on pituitary functions and other reproductive parameters are still not fully understood in buffaloes. Problems related to estrus detection constitute a major constraint to increase reproductive rates in the water buffalo (Shah *et al.*, 1990; Barkawi *et al.*, 2003 and Abdel-Ghani, 2005). This consideration indicates a need for estrus synchronization using fixed time insemination for implantation of breeding programs in buffaloes. To date, the most common synchronization schemes in buffaloes are limited either to premature regression of the corpus luteum (CL) by injection of PGF2 α or its synthetics analogues (Singh and Dabas, 1998; Abol-Roos and Gaffar, 2000 and Brito *et al.*, 2002) or by prolonging the life span of the CL by progesterone (Subramaniam and Devarajan, 1991; Luthra *et al.*, 1994; Pursley *et al.*, 1995 and Barile *et al.*, 2001). The difficulty with these approach programs in non-cyclic buffaloes has not been fully studied. The Ovsynch, developed in cattle (Pursley *et al.*, 1995), has been recently practiced in nulliparous and multiparous (Presicce *et al.*, 2005), lactating and non lactating and cyclic and non cyclic buffaloes (Paul and Prakash, 2005). The conception rate (CR) of this program is still, however, extremely low in the non cyclic buffaloes (DeRensis *et al.*, 2005). The reduced CR may be attributed to a true deep acyclic condition that is characterized by an absent or strongly reduced follicle turnover. Therefore, attainment of an adequate size of a dominant follicle required for responsiveness to GnRH may not be reached. Another explanation is that animals may be unresponsive to prostaglandin administration due to insufficient or absent luteal tissue. The administration of GnRH and GnRH analogues induces an acute release of gonadotropin in cattle (Martinez *et al.*, 2003) and buffaloes (Rastegarnia *et al.*, 2004). The effect of exogenous GnRH or equine chorionic gonadotropin (eCG) is essential to enhance the reproductive performance of buffaloes. The incidence of anoestrus in buffaloes ranges from 20 to 80 % depending on seasonal factors. In cattle High concentrations of plasma LH and FSH following treatment with GnRH are typically greater than concentrations associated with pre-ovulatory gonadotropin surge but the release of gonadotropin, however, is of shorter duration (Martinez *et al.*, 2003). The injection of GnRH induces ovulation for follicles that are at the appropriate maturational stage and possess the revealant LH receptors (Xu *et al.*,

1995). In buffaloes, treatment with GnRH has been shown to induce ovulation that is accompanied by the appearance of luteal tissue on the ovary within 14 h and increased circulating concentrations of progesterone within 96 h (Campanile *et al.*, 2008). The aim of the present study was to characterize the pituitary and ovarian response to treatment with a GnRH on day 12-14 postpartum in buffaloes. Attention was directed also to investigate the potency of GnRH to induce ovulation, formation of an accessory CL and increase circulating concentration of progesterone. It was also aimed to study the effect of GnRH on ovarian dynamics, hormonal profile and conception rate in cycling buffalo-cows.

MATERIALS and METHODS

1. Animals, management and experimental design

The current study was conducted on twenty four, apparently healthy, lactating buffalo-cows (*Bubalus bubalis*) in their second to fifth parity during the period from April to November, 2013 in Beni-Suef Province, Egypt. The animals were fed ad libitum with seasonal green fodder and wheat straw. In addition, 1 kg of concentrates was given for each kilogram of milk produced. The buffaloes had calved 12-14 days before the start of the treatment. Calves from these buffaloes were allowed to suckle for 5-10 min. before and after each milking, twice daily. The buffaloes were hand milked twice daily, at early morning and at evening. The animals had undergone a general veterinary assessment of their reproductive tract and ovaries by rectal palpation and none had any apparent abnormality. The animals were divided into two groups, group I (n=10) considered as control group without any treatment and group II (n=14) received 100 μ g GnRH, (Receptal[®]) from Schering- Plough, MSD Animal Health, is a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ, USA for each animal during the period from 12-14 days postpartum because the pituitary sensitivity, as reflected by LH response to synthetic GnRH, is suppressed in the immediate postpartum period and increases gradually by time (DeRensis *et al.*, 1993) till recovery on day 14 after calving (Alam and Dobson, 1987). Estrus detection was done by close observation from day 6 postpartum, buffaloes observed carefully in the early hours of the morning, the late hours of the evening and at 4-5h intervals during the day (Wattiaux, 1995). All animals were inseminated artificially by good quality semen and at the optimum time during estrus.

2. Ovarian and uterine examination by Ultrasonography

Ovarian structures (follicle and CL) and uterine changes were monitored daily, from day 6 postpartum until detection of the third postpartum

ovulation, using a real-time ultrasound scanner, equipped with 6-8 MHz rectal transducer probe (Pie-medical, Netherland) The day of ovulation was confirmed by the disappearance of the largest follicle followed by the formation of CL and increase in plasma progesterone concentrations. The diameters of ovarian follicles and CLs were recorded. Dominant follicle was recorded as ovulatory or non ovulatory follicle.

3. Reproductive parameters

Days from parturition to the first, second and third postpartum ovulations were calculated. Functional lifespan of CL was estimated from the day of ovulation until the day in which progesterone concentration declined for 1-2 ng/ml from the previous day, followed by the progressive decline of progesterone (regression of CL). Average diameter of CL, throughout estrous cycle, was estimated from the initial detection of CL by ultrasound to the time that CL diameter decreased for 2 mm from the previous day and continued to decline progressively. Average progesterone concentrations, throughout reproductive cycle, were calculated from the initial rise until return to the basal progesterone concentrations (0.2 ng/ml). In addition, the time from parturition till conception (days open), number of services per conception (NSC) and conception rate (CR) were recorded.

3. Estimation of progesterone concentrations (P₄)

Blood samples were collected via the coccygeal vein from day 6 post treatment and every 3 days until detecting the third postpartum ovulation. Collection was completed in clean dry and sterilized plastic test tubes and left overnight then centrifuged at 1500×g value for 10 minutes to separate the serum. The harvested serum was stored at -20 °c until assayed for P₄ concentration. P₄ concentration in serum was determined using ELISA technique, using the specific kits (EIA-1561, DRG diagnostics Instrument GmbH, Germany). The sensitivity of the assay was 0.045 ng / ml, the intra and inter-assay coefficient of variation were 6.99 % and 9.96 % (Less than 10%).

4. Estimation of FSH & LH Profiles

An LH pulse was defined as a rise in serum concentration of the hormone, two consecutive samples greater than the mean of the two previous samples, when the value of at least one of the peak samples exceed the mean by more the twice the intra-assay coefficient of variation (McNeilly and Baird, 1983). Blood samples were collected via the coccygeal vein at 0 h, 30 min, 1 h, 2 h, 3 h, and 4 h following GnRH treatment in a clean dry and sterilized plastic test tubes and left overnight then

centrifuged at 1500×g value for 10 minutes to obtain the serum and stored at -20 °c until assayed for FSH & LH concentration. Interval from GnRH injection to start of hormone rise (hours), Interval from GnRH injection to peak value (hours) and peak concentration (ng/ ml) of FSH & LH were determined by ELISA (Bovine FSH and LH from USDA Reproduction Lab., Beltsville, MD, USA was used as antigens for preparation of their specific chicken antisera. The procedure was done on three steps, 1) preparation of bovine FSH and LH chicken antisera. 2) Titration of peroxidase labeled goat anti-chicken gamma- globulin, antisera and antigen containing solutions. 3) Estimation of FSH and LH levels by the indirect ELISA (Voller *et al.*, 1979). The sensitivity of the assay was 0.24 ng / tube, the intra and inter-assay coefficient of variation were 5.3% and 9.6% (Less than 10%) for LH. While, the sensitivity of the assay was 12.0 pg FSH / tube and the intra- and inter-assay coefficient of variation were 10% and 12%, respectively for FSH.

5. Statistical analysis

Throughout the experimental study, the obtained data was subjected to statistical analysis, serum progesterone concentrations were analyzed by least square analysis of variance using GLM procedure. All values are expressed as mean ± SE relative to the reference standards. The analysis of circulating FSH and LH concentrations were performed using the MIXED procedure (SAS, 2004). Conception rate and interval from calving to first, second and third ovulation were analyzed by least square analysis of variance (SAS, 2004).

RESULTS

1. Follicular wave dynamics

Dominant follicles with diameter ≥ 0.8 cm were detected 22 days postpartum in GnRH treated group compared to 52 days postpartum in the control group. The dominant follicle that emerged at the first postpartum follicular wave ovulated spontaneously in GnRH treated groups. In the control group, the first wave dominant follicles regressed and the dominant follicle of the third to the fifth postpartum follicular wave ovulated. All animals ovulated before Day 45 postpartum in GnRH treated groups; whereas, none of the cows in control group ovulated within this period of time. The number of follicular waves from calving to the third ovulation had occurred in a short period of time in GnRH treated group compared to control group. Interval from calving to the first, second and third ovulations in GnRH treated group were significantly shorter than in control group (Table 1).

Table 1: Effect of GnRH administered on day 12-14 postpartum on ovarian dynamics in buffalo-cows

Treatment	Control group	GnRH treated group
Number	10	14
No. of FW from parturition till third ovulation	6.3±0.7 ^a	7.4±0.3 ^a
No. of buffaloes ovulated from the first FW	0/10 (0%)	10/14 (71.4%)
No. of buffaloes ovulated before Day 45 postpartum	0/10 (0%)	14/14 (100%)
Days from calving to the first ovulation	65.1±5.8 ^a	23.6±1.4 ^b
Days from calving to the second ovulation	87.6±6.4 ^a	45.2±2.3 ^b
Days from calving to the third ovulation	119.2±6.3 ^a	67.8±1.9 ^b

Within the same row, values with different superscript letters (a, b) are significantly different (P <0.05). FW = Follicular Wave.

3.2. Mean diameter of corpus luteum, progesterone concentrations and FSH& LH profiles

The diameter of CL of the first and second cycles was similar in treated group but the diameter of CL increased from the first cycle to the second cycle in the control group. The mean serum progesterone (P₄) concentrations were significantly higher in treated group in the first and the second cycle than in the control group. P₄ concentration remained at the lowest level (< 0.5 ng/ml) until day 21 postpartum in both treated and control groups, followed by an increase more than 1.0 ng/ml on day 26 postpartum

in treated group. In the control group, the P₄ rise above 1.0 ng/ml was observed later on day 60 postpartum indicating a delay in onset of luteal activity (Table 2). In both treated and control groups, while the peak LH was significantly higher in treated group in comparison with in control group (Table 3). Induced LH release started 0.5 hour after GnRH administration in both treated and control groups. Peak LH release reached within 2 hours after treatment in both treated and control groups (Table 3). The injection of a GnRH agonist also induced an increase in serum FSH concentrations at 3h compared to the control group (Table 3).

Table 2: Effect of GnRH administered on day 12-14 postpartum on the diameter of CL and P₄ concentration in buffalo-cows

Treatment	N	Diameter of CL (cm)		P ₄ concentration (ng/ml)	
		First cycle	Second cycle	First cycle	Second cycle
Control group	10	1.4 ± 0.4 ^a	2.1 ± 0.15 ^a	1.8 ± 0.3 ^{a*}	3.1 ± 0.6 ^{a**}
GnRH treated group	14	1.9 ± 0.3 ^a	2.0 ± 0.5 ^a	3.6 ± 0.2 ^b	4.1 ± 0.1 ^b

Within the same column, values with different superscript letters (a, b) are significantly different (P <0.05). Within the same row, values with different superscript asterisks (*, **) are significantly different (P <0.05). N = number of animals

Table 3: Effect of GnRH administered on day 12-14 postpartum on FSH& LH release

Parameter	Control group (N=10)		GnRH treated group (n=14)	
	FSH	LH	FSH	LH
1-Interval from GnRH injection to start of hormone rise (hours)	0.5 ± 1.3	0.5 ± 6.4	0.5 ± 0.4	0.5 ± 3.2
2--Interval from GnRH injection to peak value (hours)	3.0 ± 2.1	1.8 ± 3.6	3.5 ± 2.1	2.0 ± 5.6
3- peak concentration (ng/ ml)	15.3 ± 0.8 [*]	12.8 ± 1.9 ^a	21.5 ± 1.7 ^{**}	21.4 ± 3.8 ^b

Within the same row, values with different superscript letters (a, b) used for LH release are significantly different (P <0.05).

Within the same row, values with different superscript asterisks (*, **) used for FSH release are significantly different (P <0.05). N = number of animals

3.3. Effect of GnRH administered on days 12-14 postpartum on reproductive performance in buffalo-cows

The actual intervals from calving to first behavioral estrus and the actual interval from calving to conception (days open) were significantly shorter in the treated group than the control group. In addition the conception rate was higher in treated group than in control group (Table 4).

Table 4: Effect of GnRH administered on day 12-14 postpartum on reproductive performance in buffalo-cows

Reproductive criteria	Control group	GnRH treated group
Number	10	14
Postpartum uterine involution(days)	31.6 ± 0.2 ^a	28.3 ± 0.7 ^a
First behavioral estrus (days)	65.2 ± 3.4 ^a	42.6 ± 1.8 ^b
Number of services per conception	2.4 ± 0.8 ^a	1.3 ± 0.2 ^b
Days open	100.4 ± 2.6 ^a	86.9 ± 1.6 ^b
Conception rate	55.8 ± 2.6 ^a	77.4 ± 1.9 ^b

Within the same row, values with different superscript letters (a, b) are significantly different (P <0.05).

DISCUSSION

The poor reproductive performance in postpartum buffaloes could be partly due to suckling, which is prevalent in developing countries as it inhibits ovarian activity by affecting gonadotropic release in cows (Carruthers and Hafs, 1980). Pituitary sensitivity, as reflected by LH response to synthetic GnRH, is suppressed in the immediate postpartum period and increases gradually by time (DeRensis *et al.*, 1993) till recovery on day 14 after calving (Alam and Dobson, 1987). GnRH administration has been shown to reduce the number of small luteal cells (Helmer and Britt, 1987 and Farin *et al.*, 1988) and increase the number and diameter of large luteal cells and consequently surface area and volume of the CL resulting in positive effect on plasma progesterone levels (Chaikhun *et al.*, 2010 and Ramoun *et al.*, 2012). The gonadotropin response to the exogenous GnRH analogues treatment observed in pluriparous buffaloes in the present study was similar to that observed in cattle, under such circumstances, plasma LH level was 6 to 8 fold elevated at 30 min and 3 h after injection of GnRH (Martinez *et al.*, 2003).

Injection of 20 µg of buserelin acetate induced an LH peak between 90 and 165 min in suckled and non-suckled Murrah buffaloes (Singh *et al.*, 2006). Thus, an exogenous GnRH analogues injection causes an elevated LH and FSH levels both in cattle (Martinez *et al.*, 2003) and buffaloes (Singh *et al.*, 2006), during shorter duration compared to the pre-ovulatory level. Furthermore, suckling affected the GnRH induced LH release in buffaloes both basal level (< 1.0 ng /ml) or elevated one (> 1.0 ng/ml) (Singh *et al.*, 2006). Plasma progesterone concentration appeared to reflect the suckling induced suppression of GnRH induced LH release. Progesterone appeared to suppress LH through reducing pituitary sensitivity to GnRH and was also found to block the stimulatory effects of estradiol on

GnRH induced LH release by bovine anterior pituitary cell cultures (Padmanabhan and Convey, 1981). In the present study buffaloes that ovulated after treatment with GnRH analogues on days 12- 14 postpartum showed a progressive increase in progesterone concentration in the first and second cycles compared to the control group. Ovulation in buffaloes occurred within 48 hrs of administrating GnRH and progesterone concentration elevated within 96 hrs (Rastegarnia *et al.*, 2004). The interval from calving to complete uterine involution was not affected by administration of GnRH. It has been suggested that uterine involution is more influenced by other variables such as parity, nutrition, calf birth weight, twinning, dystocia, pathology, genetics, season and individual variability (El-Din Zain *et al.*, 1995). However, it can be delayed by persisting subclinical bacterial infection of the postpartum uterus, which can, in turn, delay the occurrence of first postpartum estrus and prolong the service period in buffaloes (Usmani *et al.*, 2001). In the current study, both treated and control groups showed uterine involution within the range reported in literature (28 to 39 days (Perera *et al.*, 1984). Moreover, the interval from calving to the first ovulation occurred earlier in GnRH treated buffaloes than in control group. However, this interval in the control group was relatively similar to those previously reported (McDougall *et al.*, 1995 and Tanaka *et al.*, 2008). Moreover, the number of follicular waves, from parturition to the third ovulation postpartum, was similar in GnRH treated buffaloes and control group. However, the interval from parturition till the third postpartum ovulation was significantly shorter in GnRH treated group than in control group. The obtained data revealed that not only the first postpartum ovulation occurred earlier but also similar number of follicular waves occurred in shorter period of time in GnRH treated buffaloes compared to control group. Accordingly, it could be concluded that GnRH hastened the first postpartum ovulation

leading to multiple cycles in short period of time prior to the first service in buffalo-cows. Moreover, the mean progesterone concentrations were lower in the first cycle compared to the second cycle of the control group. This confirms previous study (Rajamahendran and Taylor, 1990) in which plasma progesterone concentration was significantly lower in the first cycle compared to the second cycle in postpartum cows. The lower progesterone concentration in the control group might be due to the smaller CL diameter established following spontaneous ovulation. In contrast, GnRH treated group, showed no difference in progesterone concentration or CL diameter between the first and second cycle. This could be due to evidence revealed by in vitro experiments suggesting that GnRH enhances the development and production of progesterone by granulosa cell (Kuran *et al.*, 1996 and Liua *et al.*, 2003) as one of the major cells differentiated to luteal cells (O'Shea *et al.*, 1989 and Lei *et al.*, 1991). In the present study, the incidence of first postpartum behavioural estrous cycle was shorter in Gn-RH treated group than in control one. Short estrous cycle resulting from premature luteolysis is a common documented phenomenon in postpartum cows (Eger *et al.*, 1988). Strategies that stimulated dominant follicle growth before ovulation were associated with increased ovulation rate, enhanced CL development, and greater capacity of P₄ production, which are related to maintenance of pregnancy and improved fertility in buffalo (Carvalho *et al.*, 2013).

CONCLUSION

From the current study we concluded that, GnRH treatment on days 12-14 postpartum enhance the first wave follicle development and early ovulation in buffalo- cows, thus enhance the postpartum behavioral estrus, shorten number of days open, decrease number of services per conception and improve the conception rate.

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ديناميكية المبيض، معدلات الإباضة والهرمونات التناسلية الناتجة عن المعالجة المسبقة في مرحلة ما بعد الولادة باستخدام الهرمون المحرر للحاثة المنسلية في اناث الجاموس الحلاب

بكار رمضان عبد الحليم، نرمين عاطف حلمي

Email: drbakarwa@yahoo.com

كان الهدف من هذه الدراسة هو معرفة تأثير الهرمون المحرر للحاثة المنسلية في فترة ١٢-١٤ يوما بعد الولادة على ديناميكية المبيض، معدلات الإباضة، الهرمونات التناسلية ومعدلات الحمل في اناث الجاموس الحلاب. وقد أجريت الدراسة على أربع وعشرين جاموسة، خالية من الأمراض التناسلية، في موسمها التناسلي من الثاني الى الخامس. تم تقسيم الحيوانات إلى مجموعتين؛ المجموعة الأولى (ن = ١٠) تعتبر المجموعة الضابطة التي لم تتلق أي علاج والمجموعة الثانية (ن = ١٤) التي تم حقنها (١٠٠ ميكروجرام ريسينال لكل حيوان خلال الفترة من ١٢-١٤ يوم بعد الولادة). تم متابعة نشاط المبايض يوميا، من اليوم السادس بعد الولادة حتى حدوث التبويض الثالث بعد الولادة، وذلك باستخدام الموجات فوق الصوتية. تم جمع عينات الدم من اليوم السادس بعد العلاج كل ٣ أيام وحتى ثبوت التبويض الثالث بعد الولادة لقياس تركيز هرمون البروجسترون وهرمون الحاثة اللوتينية (الهرمون المحفز للإباضة) وهرمون الحاثة الجريبية في الدم. اثبتت النتائج في هذه الدراسة ان جميع الحيوانات حدث بها إباضة قبل يوم ٤٥ بعد الولادة في المجموعة المعالجة (١٤ / ١٤، ١٠٠ %). في حين أن أيا من الأبقار في المجموعة الضابطة لم تحدث تبويض خلال هذه الفترة. وكان الفاصل الزمني بين الفترة من الولادة إلى أول إباضة ومن أول إلى الثاني والثالث إباضة في المجموعة التي تلقت العلاج أقصر بكثير (١٤ ± ٢٣,٦، ٣ ± ٤٥,٢ و ٩ ± ٦٧,٨ يوما على التوالي) مما كانت عليه في المجموعة الضابطة (٨ ± ٥,١، ١ ± ٦٥,٤، ٦ ± ٨٧,٦ و ٣ ± ١١٩، يوما) على التوالي. وقد اظهرت تركيزات هرمون البروجسترون في الدم ارتفاع ملحوظ في المجموعة التي تلقت العلاج في الدورة الأولى والدورة الثانية (٢ ± ٣,٦ و ١ ± ٤,١ نانوجرام / مل) مما كانت عليه في المجموعة الضابطة (٣ ± ١,٨ و ٦ ± ٣,١ نانوجرام / مل) على التوالي. أما تركيزات هرمون الحاثة اللوتينية (الهرمون المحفز للإباضة) فكانت مماثلة في كل من المعالجة والمجموعة الضابطة، في حين أن ذروة هرمون الحاثة اللوتينية (الهرمون المحفز للإباضة) أعلى بكثير في المجموعة التي تلقت العلاج (٨ ± ٣,١ نانوجرام / مل) من المجموعة الضابطة (٩ ± ١٢,٨ نانوجرام / مل). كما اظهرت نتائج هذه الدراسة ان الفترة الفعلية من الولادة لأول دورة شبق والفترة من الولادة حتى ثبوت الحمل كانت أقصر بشكل ملحوظ في المجموعة التي تلقت العلاج (٨ ± ٤٢,٦ يوم، ٦ ± ٨٦,٩ يوما على التوالي) من المجموعة الضابطة (٤ ± ٣,٤ ± ٦٥,٢، ٦ ± ١٠٠,٤ يوما على التوالي). كان معدل الحمل اعلى في المجموعة التي تلقت العلاج (٩ ± ٧٧,٤) مقارنة مع المجموعة الضابطة (٦ ± ٥٥,٨). من الدراسة الحالية نخلص إلى أن العلاج بالهرمون المحرر للحاثة المنسلية في يوم ١٢-١٤ بعد الولادة يساعد على تحفيز الإباضة في وقت مبكر في اناث الجاموس، وبالتالي تحفيز دورة الشبق بعد الولادة، وتقصير الفترة من الولادة حتى ثبوت الحمل، وانخفاض عدد التفقيحات اللازمة للاخصاب وتحسين معدل الحمل.