Hormonal Changes During Busserlin (GnRH) Priming Regimen for Superovulation in the Camel (*Camelus dromedarius)*

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Abstract:

Seven mature non-pregnant female camels (*Camelus dromedarius)* have been used in this study. The female camels were injected with 20 µg. buserlin at the first day of the experiment, then scanned for ovarian activities. Superovulatory treatment commenced when a minimal ovarian activity was observed. Superovulatory treatment consisted of a combination of follicle stimulating hormone (FSH) and equine chorionic gonadotrophin (eCG) . One dose of 2000IU eCG was intramuscularly injected for all female camels, but 50mg FSH was splitted and injected twice daily for 4 consecutive days in a decreasing manner. The female camels were twice mated (12 hours apart) with fertile male camels when the ovarian follicles are considered sufficiently mature at 1.3 to 1.9 cm in diameter. Each female camel received 20 µg buserlin at the time of the first mating. All experimental camels responded to the superovulation regimen. The lowest response was 7Corpora lutea, while the highest response was 20Corpora lutea. The over all average of the number of ovarian corpora lutea was 13.43±1.77. The number of anovulated follicles ranged from 0 to 2 with an average of 1.57±0.28. Plasma progesterone concentrations showed an increase from 2.21 ± 1.01 ng/ml before GnRH injection to 4.33 ± 0.96 ng/ml at the time of superovulatory treatment. A sharp increase in plasma progesterone concentration to 18.2 ± 4.78 ng/ml was measured 8 days post-mating. Plasma estradiol 17 ß concentration was nearly constant throughout the experimental period. It fluctuated around 22.00 pg/ml before GnRH treatment and 18.00 pg/ml 8 days post-mating. A highly significant negative correlations ($P<0.01$) was observed between the ovarian response and the plasma estradiol ($r= -0.84$) concentration before initiation of superovulation. There was a significant ($P<0.05$) correlation coefficient between ovarian response and the plasma progesterone concentrations ($r= 0.73$) at the day of recovery.

In conclusion, the current regimen of camel superovulaton produced a promising superovulatory response. The key for such result is the use of a combination of eCG and FSH as a superovulatory agent injected when there is no follicular activity. Absence of follicular activity must be confirmed by ovarian scanning and plasma estradiol assay.

Introduction:

The opportunities of improving camel reproductive efficiency are limited due to the continued use of traditional systems of reproductive management in most breeding herds (Cooper *et al*.,1990). The use of embryo transfer (ET) technique in the camel breeding industry can be of a particular value to increase the number of progenos from desirable male and female genetic combinations, whether this be for racing or production of meat or milk (Yagil and Van Creveled,1990). Moreover, ET could provide more progeny from subfertile camels and those calving late in the breeding season. It could also be used to test techniques such as artificial insemination with fresh, cooled or frozen semen (Mc Kinnon *et al*.,1994).

The fundamental objective of superovulation is to increase the number of fertile eggs given by the treated outstanding female. Optimum superovulatory response in farm animals achieved when the superovulatory hormone was administered during the midluteal phase of the cycle which was subsequently curtailed one to three days later by prostaglandin hormone (Lerner *et al*.,1986; Ismail, 1991). However, superovulation is a challenge in camels as they are induced ovulators and corpus luteum is only developed when mating occurs (Al-Eknah,2001). Superovulation in the camel, therefore, was obtained by injecting eCG or FSH hormones at the end of induced luteal stage which provided by progesterone releasing intravaginal device (PRID), controlled intravaginal device release (CIDR) or daily progesterone injection (Anouassi and Ali,1990; Skidmore et al.,1992; Ismail et al.,1993; Mc Kinnon *et al*.,1994; Tinson et al.,2000; Ismail et al.,2006; Ismail and Al-Eknah,2006).However, attempts of superovulation in the camel have generally yielded poor and inconclusive results (Purohit,1999)

It is of great interest in embryo transfer programs, prior to induction of superovulation, to predict the non- or poor responsive donors. This,

perhaps, would contribute to substantial savings of financial and time resources. In cattle and buffaloes, several studies (Landgren *et al*.,1982; Goto *et al*.,1988; Ismail *et al*.,1992a,b) have shown an intimate relationship between plasma progesterone and estradiol levels at start of superovulation and the superovulation response. Moreover, ovulation rate, embryo production and embryo quality can be predicted by the plasma levels of these hormones (Saumande *et al*.,1985; Tamboura *et al*.,1985; Goto *et al*.,1987). In camels similar information are scanty.

The present research aims to explore a different regimen for camel superovulation. In addition, plasma progesterone and estradiol concentrations are measured during different periods of the regimen. The relationship between the plasma concentration of these hormones and the superovulatory response is considered.

Materials and Methods:

1. Camels

Seven mature non-pregnant female camels (*Camelus dromedarius)* were used in the present study. They were 6 to 14 years old, kept in open yard and fed on barley and rhodes grass hay. Water was provided ad libitum. Camels were kept in the Camel Research Centre, King Faisal University , during the experimental period.

2. Priming and Superovulation

Experimental camels were examined by ultrasonography before commencement of any injection .All showed follicular activities of different sizes. All camels were injected with 20 µg. buserlin (Receptal^R, Intrervet Ltd., Holland) at the first day of the experiment. 7 days later, the camels were scanned for ovarian activities. Superovulatory treatment started when a minimal ovarian activity was observed. Superovulatory treatment consisted of a combination of FSH (FSH-P^R,Sigma, USA) and eCG (Folligon^R, Intrervet Ltd., Holland) hormones. Regimen of superovulation is presented in Table1.

3. Mating and ovulation

The development of ovarian follicles was monitored by ultrasound scanner. Scanning was daily performed for all camels starting the next day

of terminating the superovulatory hormone until the majority of follicles were considered sufficiently mature (1.3 - 1.9 cm in diameter), where mating with fertile male camels was allowed twice 12 hours interval. Each female camel received 20 µg buserlin just at the time of first mating.

Table (1)
Regimen of superovulation in the experimental camels

Day of experiment	Event
1	Injection of 20 µg buserlin
7 (With minimal ovarian activity)	Am 2000IU eCG + 4ml FSH Pm 4ml FSH
8	Am 3ml FSH Pm 3ml FSH
9	Am 2ml FSH Pm 2ml FSH
10	Am 1ml FSH Pm 1ml FSH
14	Mating + Injection of 20µg buserlin
22	Evaluation of the ovarian response

4. Evaluation of the ovarian response

The ovarian response of the experimental camels was evaluated 8 days post-mating (7 days post-ovulation). Evaluation was conducted by rectal palpation and ultrasonography. Two camels were subjected to laparotomy for more accurate counting of the excessive number of corpora lutea.

5. Hormonal assay

Blood samples were collected into heparinized tubes by jugular venipuncture just before GnRH treatment throughout until the time of mating. Plasma samples were stored at -20°C until hormonal analysis. Plasma estradiol -17ß was measured according to the method adopted by Landgren et al.(1982) whereas plasma progesterone was measure using the method employed by Sheehan *et al*. (1982). Both hormones were measured using Coat-A-count kits (Diagnostic Products Corporation,USA). Gamma counter (Berthold) was used for counting and the produced number was converted by the way of calibration curve for

measuring both hormones in unknown samples. The sensitivity of the assay defined as the smallest concentration significantly ($P<0.05$) distinguishable from zero was 0.1ng/ml and 3pg/ml plasmas for progesterone and estrogen hormones, respectively. The intra-and inter assay coefficients of variation were 7.9 and 8.5% for progesterone and 12.3 and 16.8% for estrogen hormones, respectively.

6. Statistical analysis

Data were expressed as mean ± SEM. Correlation coefficient was used to correlate between the ovarian response and both of plasma progesterone and estradiol concentrations. Statistical analysis was performed according to Snedecor and Cochran (1979).

Results:

1. Superovulatory response

All experimental camels (100%) responded to the current regimen of superovulation The lowest response (7Corpora lutea) was observed in camel number 2 while the highest response (20Corpora lutea) was recorded in camel number 7 (Table2).The over all average of the number of ovarian corpora lutea was estimated to be 13.43±1.77.The number of anovulated follicles ranged from 0 to 2 with an average of 1.57±0.28 (Table 2)**.**The ovulation rate was estimated to be 89.52%.

2. Hormonal analysis

Plasma progesterone concentrations showed an increase from 2.21 ± 1.01 ng/ml before GnRH injection to 4.33 ± 0.96 ng/ml at the time of superovulatory treatment. A sharp increase in plasma progesterone concentration to 18.2 ± 4.78 ng/ml was measured 8 days post-mating. Plasma estradiol 17ß concentration was nearly constant throughout the experimental period. It fluctuated around 22.00 pg/ml before GnRH treatment and 18.00 pg/ml 8 days post-mating (Table, 3).

3.Hormonal-ovarian response relationship

Correlation coefficients between the ovarian response and both of plasma progesterone and estradiol concentrations are shown in table (4). Just before initiation of of the superovulatory treatment, a highly significant negative correlations ($P<0.01$) was observed between the ovarian response

and the plasma estradiol (r= -0.84) concentration. However, the correlation between the ovarian response and plasma progesterone concentration was non-significant (r= -0.60) .At the day of recovery the correlation coefficient between ovarian response and the plasma progesterone concentration (r= 0.73) was significant (P<0.05). However, the correlation coefficient between ovarian response and the plasma estradiol concentration (-0.23) was non-significant .

Discussion:

The regimen of superovulation used in the current study based upon priming the donor camels with GnRH followed by daily scanning of the ovaries for six successive days. On day seven postGnRH treatment, when there was no follicular activity a combination of eCG and FSH-P was injected. It was planned to overcome any follicular activity present before initiation of the

superovulation by daily treatment with 100 mg progesterone in sesame oil until disappearance of the follicular structure.However, all experimental camels had no follicular structyres at commencement of superovulation. It is evident that the key for

Table (2)

The ovarian response of the experimental camels (Mean ±SEM)

Animal Number	**Number of corpora lutea (Corpora lutea)**			**Number of follicles**		
	L	R	Total	L	R	Total
5	6	3	9	-	2	2
6	8	10	18	1	-	1
2	4	3	7	1	1	2
3	6	3	9	1	1	2
1	5	10	15	-	-	-
4	4	11	16	-	2	2
7	6	13	20	-	2	2
mean	1.57 ± 0.28			13.43 ± 1.77		

Table (3)
Plasma progesterone and estradiol 17ß concentrations during the different phases of the superovulatory regimen in the experimental camels (Mean ±SEM)

Time of plasma sampling	Progesterone Concentrations (ng/ml)	Estradiol 17ß Concentrations (pg/ml)
Just before GnRH treatment	2.21±1.01	22.00±1.03
At superovulatory treatment	4.33±0.96	18.75±1.84
At day of recovery	18.2±4.78	22.09±1.21

Table (4)
Correlation coefficients (r) between ovarian response and Plasma progesterone and estradiol concentrations during the different phases of the superovulatory regimen of the experimental camels (Mean ±SEM).

Ovarian response	Progesterone concentration (ng/ml)	Estradiol 17ß concentration (pg/ml)
Just before initiation of the superovulatory treatment	-0.60	-0.84**
At the day of recovery	0.73*	-0.23

**Significant at P<0.01 *Significant at P<0.05

successful superovulation is the absence of ovarian follicles at the start of superovulatory treatment. This concept was supported in the current study by the significant negative correlation (P<0.01) between the ovarian response and the plasma estradiol concentration at start of the superovulatory treatment. Similar findings were reported by Tibary and Anouassi (1997); Skidmore (2000); Ismail *et al*.,(2006); Ismail and Al-Eknah (2006) who found that the best stimulation of the camel ovaries occurred when treatment with exogenous gonadotrophic hormone started at minimum follicular activity. The presence of follicular activities at the time of treatment is probably associated with high estrogen level which blocked the release of pituitary FSH and cause low ovarian response (Saumande, 1980)

The response of all experimental camels (100%) to the superovulatory treatment and the high superovulatory response in the present work are considered promising results. High incidence of non-responsive camels to superovulation (developed less than 3Corpora lutea) was a problem reported by many investigators. Skidmore (2000) estimated an incidence of 20-30% non-responsive camels. The author attributed this result to immunization of some females against the superovulatory hormone. The high incidence of non-responsive camels was also noted by Cooper *et al.* (1992); Mc Kinnon and Tinson (1992); Vyas (1998); Ismail *et al.* (2006). Moreover, it seemed that this high incidence did not influenced by the physiological status of the camels whatever they were dry or lactating (Ismail and Al-Eknah, 2006) .

The superovulatory response of the experimental camels averaged 13.43±1.77 CL with a range of 7.00 to 20.00 CL.This ovarian response is much higher than the means of 4.6 to 5.7 CL reported by several authors (Skidmore *et al.,*1992; Ismail *et al*.,1993; Vyas,1998) injected the superovulatory hormone after 7 days progesterone priming via PRID or CIDR. The mean number of Corpora lutea obtained herein, is also higher than the means of 8.75 to 9.83 CL developed when the superovulatory hormone injected at the end of 10-15 days progesterone treatment (Mc Kinnon et al.,1994; Tinson et al.,2000; Ismail et al.,2006; Ismail and Al-Eknah,2006) . However, the high incidence of responsive camels to superovulation and the high ovarian response in this study are probably related to the regimen of superovulation, which guaranteed absence of follicular activity at treatment, and the use of combined eCG and FSH in the superovulation process. In this aspect, Skidmore (2000) stated that the best ovarian response in camels was seen when a combination of both eCG and FSH was used in the ovarian stimulation.

The increase of mean plasma progesterone concentration of the experimental camels from 2.21±1.01ng/ml before GnRH treatment to 4.33 ± 0.96ng/ml at superovulatory treatment coincided with the decrease of plasma estradiol concentration at the same periods. This may indicates the changing of the follicular structures into luteal structures as a result of GnRH treatment. This is justified by the level of plasma progesterone

which was in the range of 2.5-4.5 ng/ml that reported in ovulated camels (Skidmore *et al.,*1997). However, the sharp increase of progesterone at day of recovery is probably associated with the increased number of corpora lutea. It was found that the low levels of progesterone at the day of recovery indicated premature regression of corpora lutea (Jensen *et al.,*1982; Lindsell *et al*.,1986).

The highly significant ($P<0.01$) negative correlation between the ovarian response of the experimental camels and plasma estradiol concentration at the start of superovulation coincided with the results of ovarian scanning at such time which revealed absence of follicular activity. Skidmore(2000) reported that if follicles are present at the time of treatment, these tend to develop into overlarge follicles before the new stimulated follicles have had chance to develop. On the other hand, the relationship between ovarian response of the experimental camels and plasma progesterone concentration at the start of superovulation was non-significant. However, in many experimental camels a higher ovarian response was obtained when progesterone concentration at the start of superovulation was low. On the contrary to this observation Goto *et al*. (1987) and Ismail *et al*. (1992a) reported a significant relationship between ovarian response and progesterone concentration at the start of superovulation in cattle. Callesen *et al*. (1988) informed that higher levels of progesterone at start of superovulation in cattle tended to suppress the basal luteinizing hormone (LH) discharge from initial injection of gonadotrophin to injection of prostaglandin; this allowed for greater storage of LH and subsequently produced a broader and higher LH surge resulting in higher ovulation response. However, in camels it seemes that low progesterone level at start of superovulation may stimulates the release of gonadotrophins for higher follicle formation. Then, LH surge is released from pituitary gland 3-4 hours after mating and ovulation occurred 24 to 36 hours later (Marie and Anouassi,1987). Higher LH surge was provided by mating the donor camels twice, 12 hours apart, with injection of GnRH at the first mating.

In conclusion, the current regimen of camel superovulaton produced a promising superovulatory response. The key for such result is the use of a

combination of eCG and FSH as a superovulatory agent injected when there is no follicular activity. Absence of follicular must be confirmed by ovarian scanning and plasms estradiol assay.

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التغيرات الهرمونية المصاحبة للتبويض المتعدد المستخدم فيه الهرمون المنشط للغدة النخامية في الإبل

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الملخص :

تم أجراء الدراسة الحالية على ٧ نوق حقنت ٢٠ ميكروجرام من الهرمون المنشط للغدة النخامية في اليوم الأول للتجربه ثم تم فحص المبايض بجهاز الموجات فوق الصوتية لمتابعة نشاطها. بدأ علاج النوق لإحداث التبويض المتعدد عند أقل نشاط للمبايض. تكون علاج إحداث التبويض المتعدد من هرمونى الفرس المشيمائي المحفز للمناسل(جرعه واحده) والغدة النخامية المنشط للجريبات (جرعتين يومياً لمدة ٤ أيام). تم تلقيح النوق مرتين بينهما ١٢ ساعة باستخدام فحول خصبه عند وصول الجريبات حجم ١٣- ١٩مم. وقد استجابت كل النوق للتبويض المتعدد. وقد كان أقل عدد للأجسام الصفراء ٧ في حين كان اكبر عدد ٢٠ بمتوسط ١٣,٤٣ ±١,٧٧ جسم اصفر لكل ناقة. وقد تم قياس تركيز هرموني البروجستيرون والأستروجين خلال التبويض المتعدد.وبينت النتائج وجود ارتباط معنوي قوى سالب بين التبويض المتعدد و تركيز هرمون الأستروجين عند البدأ في إحداث العلاج للتبويض المتعدد فى حين لم يكن هناك ارتباط بين التبويض المتعدد و تركيز هرمون البروجستيرون فى نفس الفترة. كما تبين حدوث ارتباط معنوي بين التبويض المتعدد و تركيز هرمون البروجستيرون عند تقييم الاستجابة للتبويض المتعدد. وقد خلصت الدراسة أن هذا النظام للتبويض المتعدد قد أعطى نتائج مشجعة وأن السر في ذلك يكمن في العلاج بالمزج بين هرموني الفرس المشيمائي المحفز للمناسل والغدة النخامية المنشط للجريبات على أن يبدأ العلاج عند الغياب التام لجريبات المبيض، ويمكن التأكد من ذلك بالفحص بجهاز الموجات فوق الصوتية وقياس تركيز هرمون الأستروجين في الدم.