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Ovulation induction using GnRH and hCG during non-breeding season in the one humped camel (*Camelus dromedarius*)

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ABSTRACT

The aims of the present study were to monitor the ovulatory response following ovulation induction by GnRH and hCG, and determine the effect of GnRH and hCG on induction of ovulation. A total (19) one humped camels were used in this experiment and divided into three groups: Group (A) Camels (N=7) were intramuscular injected by 2 ml GnRH Group (B) Camels (N=6) were intravenous injected by 3 ml hCG. Group (C) without any hormonal treatment, camels (N=6) were intramuscular injected 1 ml by distilled water. In all groups ovulation was observed by ultrasonography. The proportion of she camels that ovulated during 24-48 hours in response to treatments were (6 / 7 vs 4 /6 vs 0 / 6) in the GnRH, hCG and the control groups, respectively. The results showed that were significant differences in the means $\pm$ SD ($P \leq 0.01$) between the treated groups compared to the control group. But there were no significant differences between the GnRH and hCG groups ($P \leq 0.05$).

INTRODUCTION

Camels are an important livestock species in the arid and semi-arid zones, it contributes significantly to the livelihood of the pastoralists and agro-pastoralists living in the fragile environments of the desert and semi-desert of Asia and Africa (Ishag, 2011).

The reproductive performance of camels is generally regarded to be low (40%), this could be due to the late age of reaching puberty (3 - 4 years), the long gestation period (13 months) and the relatively high incidence of abortions and non-conceptions possibly attributed to poor nutrition, poor management, limited breeding opportunities for the females due to seasonality of breeding (Skidmore, 2013). In addition, female camelids are induced ovulators, and therefore ovulation must be induced before insemination (Skidmore, 2013). Follicular growth occurs in regular waves during the breeding season (Fatnassi *et al*, 2014). Where waves of follicular growth,

maturation and atresia occur constantly in both ovaries (Manjunathaa *et al* 2012). Many authors were mentioned that camels reproduction is disabled by different constrains as semen Characteristics, long gestation period, late sexual puberty and maturity, limited breeding season and the mechanism of oestrous cycle and ovulation of she- camel (Deen, 2008; EL-Hassanien *et al*,2010). Females show higher breeding activity during winter and spring than summer and autumn seasons, changes in food supply, mineral supplementation and photoperiod have been suggested to be responsible for not only productive performance but also for seasonal breeding pattern of female camel, this view is supported by the observations that ovarian activity did not cease completely during summer and autumn seasons (Onjoro *et al*., 2006; and Fatnassi *et al*, 2014).

The mechanism by which ovulation is initiated has been used to classify mammals as either spontaneous or induced ovulators, based on the biological process that triggers release of Gonadotropin-Releasing Hormone (GnRH) and initiates the ovulatory cascade, mice, cattle, horses, and pigs are considered spontaneous ovulators because ovulation occurs at regular intervals as a result of increasing systemic concentrations of estradiol from growing dominant follicles, which stimulates GnRH secretion from neurons in the hypothalamus, which in turn elicits a surge release of LH from the anterior pituitary gland (Espey & Lipner 1994). In contrast, ovulation in induced ovulators does not occur at regular intervals, but rather in response to a copulatory stimulus, camels are induced ovulators and therefore normally only ovulate when mated, so it is more accurate to describe the changes in follicular development as a follicular wave pattern rather than oestrus cycle (Skidmore, 2013).

Ovulation can be induced in Dromedary and Bactrian camel by a single injection of LH, GnRH or Human Chorionic Gonadotropin (hCG) (Vyas and Sahani, 2005; Ismail *et al*, 2007 ; Moghiseh, *et al*.2008 ; Rawy, 2011; Derar *et al*, 2014). Because camels are an induced ovulating species, the ability to control ovulation is of fundamental importance (Skidmore, 2011).

The low reproductive efficiency in she camels could be improved by better understanding of the reproductive cycle and increased use of assisted reproduction techniques such as ovulation induction, artificial insemination and embryo transfer (Skidmore, 2011).

Objectives of the study

The aim of the present study was to monitor the ovulatory response following ovulation induction by GnRH and hCG, and determine the effect of GnRH and hCG on induction of ovulation.

MATERIALS AND METHODS

Area of study:

This experiment was conducted during the non-breeding season from May to June, 2014. At the Camel Reproduction Centre (CRC), Nakhlee, this located 48 km east the center of Dubai, UAE.

Experimental animals

19 Mature Dromedary she – Camels, aged 6-8 years (average weight between 500-600 kg).

Management

Animals were maintained in groups in fenced pens, each of 0.002-0.004 Km² area. They were fed a diet of commercially formulated camel rations mixed concentrates and Lucerne hay twice a day to provide their requirements. Water was permanently available, and exposed to natural day length and ambient temperatures. The animals were identified by Neck Tags (Necklace neck).

Hormonal Protocols for Ovulation Induction

The She – camels were allocated to three groups:

Group (A):

7 animals were intramuscular injected by 2 ml of GnRH {GONAVET Veyx® 10 ml}, each 1 ml contain Gonadorelin – [6-D-Phe]. Batch No: 12J252 from Veyx – Pharma GmbH D-34639 Schwarzenborn, Germany.

Group (B):

6 animals were intravenous injected by 3 ml of hCG {CHORULON® Chorionic Gonadotropin 1000 I.U} with 5 vials as diluents (Sterile Phosphate Buffer water 10 ml). Batch No: A 037A02 – Code No: 022219 from Intervet INC, Millsboro, DE 19966 Holland.

Group (C):

6 animals were intramuscular injected by 1 ml of distilled water.

Batch No: 124051, Hameln Pharmaceuticals LTD, UK.

Ovarian Examination

The ovaries of every camel were examined by ultrasonography on alternate days throughout the defined experimental periods (0 hrs. = time of injections, 48 hrs = after 48 hours to detect and follow the follicles growing and the onset of ovulation. Week 1 = after 7 days, and Week 2 = after 14 days to identify the presence and persistency of the corpus luteum on the same ovary that had previously held the dominant follicle.

Ultrasonic examination

The females restrained in a standing position in crush, the perineal region cleaned very well and the tail lifted and rolled with gauze. The rectum was evacuated from the feces after had been wearing the long gloves and putting the lubricant gel, similar to the standard technique which used in Cattle. Then the linear probe (array transducer) passed through the rectum and placed in contact with the bottom of the rectum to reflect the images from the ovary interface, which displayed in the monitor.

An ALOKA (Model Prosound 2) real time scanner with a 5 / 6 MHz linear array transducer (Hitachi Aloka Medical, Ltd, Tokyo, Japan) was used. All follicles (≥ 0.5 cm in diameter) and corpus luteum were monitored, counted and measured using the internal electronic calipers and photographed using a Sony video printer (Model UP 860CE) (Al Carmal Ltd, Dubai, UAE) interfaced with the scanner. Data recorded on the scanning worksheet.

Statistical analysis

The data were analyzed using SPSS statistical software version 16 for windows (SPSS, 2015). The Follicles size differences between groups and within groups were compared by One way ANOVA all values expressed as means $\pm$ SD, while the incidence of Ovulation compared by 2 x 2 crosstab (Chi square test).

RESULTS

Table (1) showed that there were no any significant differences between all groups in the follicle size (Diameter cm) in 0 hours to be sure that there was no effect or interaction made by the follicles on the hormonal profile or the parameters (Figure 1)

Ovulation was observed in all groups treated with Gonadotropin Releasing Hormone (GnRH), Human Chorionic Gonadotropin (hCG) and distilled water respectively. The proportion of she camels that ovulated during 24-48 hours in response to treatments were 6 / 7 vs 4 /6 vs 0 / 6 in the GnRH, hCG and the control groups, respectively (Table 2). While the Table No: 3 showed that there were significant differences between the treated groups (A and B) compared to the control group (C). But there were no significant differences between the GnRH and hCG groups ($P \leq 0.05$).

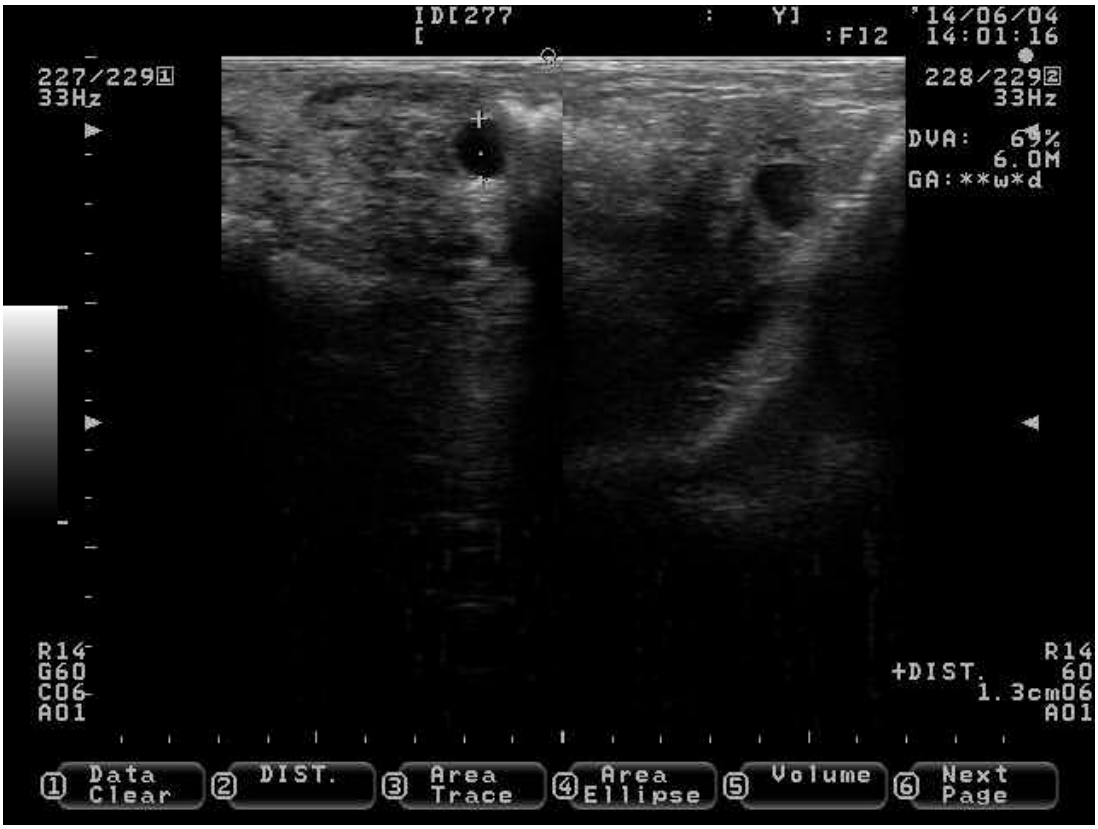

Figure 1: Measuring the Follicle size (Diameter cm)

Table 1: Follicles Size (Diameter cm) in 0 hours in different groups in she - camels

Groups	N	Means ± SD	P. Value
GnRH	7	1.46 ± 0.05	
hCG	6	1.50 ± 0.06	0.45
Control	6	1.43 ± 0.14	

* N = Number of Animals

Table 2: Ovulation response to hormonal protocols during 24 - 48 hours In She – camels

Groups	Ovulated	Ovulation %
GnRH	6 / 7	85.7 %
hCG	4 / 6	66.6 %
Control	0 / 6	0 %

Table 3: Ovulation onset during 24-48 hours in She – camels injected by hormonal protocol

Groups	Pearson Chi – Square x^2	df	P. Value
GnRH vs hCG	0.66	1	0.42
GnRH vs Control	9.55	1	0.002
hCG vs Control	6.00	1	0.014

DISCUSSION

Results of this study are consistent with those of a previous study of ovulation induction in dromedary camel done by Skidmore (2011), and supported the hypothesis that camels are induced ovulators and thus normally only ovulate in response to mating. In the absence of mating, ovarian follicles tend to regress after a period of growth and maturity, this was observed by Manjunathaa et al, (2012); Derar *et al*, (2014). While Skidmore *et al*, (1996); Tibary and Anouassi, (1996) and Tibary *et al*, (2007) reported that in the absence of mating or other ovulatory stimuli (i.e. GnRH or hCG treatment) there is a succession of over lapping follicular waves with variable rhythm showing three phases: growth, maturation and regression. In the present study the ovulation occurred during 24-48 hours from the injection of GnRH and hCG and there was highly significant difference ($P \leq 0.01$) between the GnRH and hCG compared with the control groups, respectively. Similar results were observed by a number of authors (Skidmore, 2011; and Sheldrick *et al*, 1992).

The present study indicated that ovulation may be induced 24–48 hours following treatment with (GnRH), which is in conformity with Bono *et al*, (1985); Anouassi *et al*, (1994) or its analog like Busereline which reported by Cooper *et al* (1992); McKinnon *et al* (1992); Musa *et al*, (1993) and Skidmore *et al* (1996). The present study reported that ovulation can be also induced by an administration of (hCG). Similar results were observed by Anouassi *et al*, (1994); Ismail *et al* (1993). Also agrees with Adams *et al*, (2009) they mentioned that injections of (hCG) lead to ovulation 24 hours later in *Lama pacos*.

The present study indicated that there was no significant difference ($P \leq 0.05$) between the GnRH and hCG in inducing the ovulation 6/7 (85 %) and, 4/6 (66 %) respectively, which agrees with results of Skidmore *et al* (1996). Similar results were observed by Rato *et al* (2006), they mentioned that the proportion of mice that ovulated was similar among GNRH, hCG groups, suggesting that the hypothalamo – hypophyseal axis was functional and that the ovaries were capable of responding.

Conclusions

It could be concluded and recommended that:

1. Ovulation can be induced in non-breeding season by using GnRH and hCG.
2. To increase the successes rates of ovulation induction the follicle size diameter should be between 1.0 to1.7 cm.
3. No significant differences between GnRH & hCG so we recommend to use the cheapest one.

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REFERENCES

Adams, G. P., Ratto, M. H., Collins, C. W., and Bergfelt, D. R (2009) Artificial insemination in South American camelids and wild wquids. *Therionology*. **71**: 166-175.

Anouassi, A., Tibary, A., Adnani, M., and Sghiri, A. (1994) Characterization of the preovulatory phase characterization in *Camelus dromedarius* female and induction of ovulation. In: *Proceeding of International Foundation for Science Conference*, Niamey, Niger, pp. 159–167.

Bono, G., Dahir, A.M., Comin, A., and Jumale, M.A (1985) Seasonal variations of LH response to GnRH treatment in camels (Camelus dromedarius). *Journal of Reproduction and Fertility*. **73**: 335–339.

Cooper, M.J., Skidmore, J.A., Allen, W.R., Wensvoort, S., Billah, M., Chaudhry, M.A., and Billah, A.M (1992) Attempts to stimulate and synchronise ovulation and superovulation in dromedary camels for embryo transfer. In: *Proceedings of the First International Camel Conference*,Dubai, UAE, pp. 187–191.

Deen, A. (2008). Why artificial insemination in dromedaries is not successful? *Proceeding of the WBC / ICAR, Satellite Meet. Camelid Reproduction.* Budapest, Hungary, p.28-32.

Derar, R., Ali, A., and Al Sobayil, F. A (2014) The postpartum period in dromedary camels: Uterine involution, ovarian activity, hormonal changes, and response to GnRH treatment. *Journal of Animal Reproduction Science*. **151**: 186-193.

El-Hassanien, E,E., El- Bahrawy, K, A., and Zagloul A, A(2010) Artificial insemination and ovulation in the dromedary she- camel. *Journal of Nature and Science,* **8**(9).

Espey, L., and Lipner, H (1994) Ovulation. In: *Physiology of Reproduction* (eds). Knobil, E., and Neill, J.D Vol: **1**. Raven Press, New York, p 725–781.

Fatnassi, M., Padalino, B., Monaco, D., Khorchani, T., Lacalandra, G. M., and Hammadi, M. (2014). Evaluation of sexual behavior of housed male camels (*Camelus dromedarius*) through female parades: correlation with climatic parameters. *Tropical Animal Health Production,* **46**: 313-321.

Ishag, I (2011) Camels of the Sudan, Production system, Phenotypic and Molecular Characterization. LAP LAMBERT Academic Publishing. Germany.

Ismail, S. T., Al Eknah, M., and Hemeida, N (2007) Superovulation trails for embryotransfer in the camel (*Camelus dromedarius*). *Scientific Journal of King Faisal University*. (*Basic and Applied Sciences*). **8**: 51-60.

Ismail, S.T., Azab, G.A., Abdoon, A.S.S., and El Belely, M.S (1993) New approach for superovulation and embryo recovery in the one humped camel.

VeterinaryMedicine Journal. Giza **41**: 47–52.

Manjunathaa, B. M., David, C. G., Pratap, N., Al Bulushi, S., and Hago, B. E (2012) Effect of progesterone from induced corpus luteum on characteristics of a dominant follicle in dromedary camels (*Camelus*

dromedarius). *Journal of Animal Reproduction and Science*. **132**:231-236.

McKinnon, A.O., Tinson, A.H., Allen, W.R.H.A.J., Mayhew, I.G., Snow, D.H., and Wade, J.F (1992) Embryo transfer in dromedary camels. In: *Proceedings of the First International Camel Conference*, Dubai, 2nd–6th February, pp. 203–208.

Moghiseh,A., Nisari-Naslaji,A., Nikjou, D., Germai,A., Razavi, K., and Mostafaey, M (2008). The effect of LH and GnRH analogues on induction of ovulation in bacterian camel(Camelus bactrainus). *Iranian Journal of veterinary research.* Vol:**9** No:4Ser. 25.

Musa, B., Sieme, H., Merkt, H., Hago, B.E.D., Cooper, M.J., Allen, W.R., and Jochle, W (1993). Manipulation of reproductive functions in male and female camels. *Journal of Animal Reproduction Science*. **33**: 289–306.

Onjoro, P. A., Njoka-Nijir, E. N., Ottaro, J. M., Simon, A., and Schwartz, J.J (2006) Effects of Mineral Supplementation on milk yield of free-ranging (*Camelus dormedarius*) in northern Kenya. *Aasian-Australian Journal of Animal Science*. **19**:1597-1602.

Ratto, M.H., Huanca, W., Singh, J., and Adams, G.P (2006) Comparison of the effect of ovulation-inducing factor (OIF) in the seminal plasma of llamas, alpacas, and bulls. *Theriogenology* **66**: 1102–1106.

Rawy, M. S., (M.V.Sc theisi)(2011) Follicular Dynamics and induction of ovulation in female dromedary camel. Assiut University. Assuit. Egypt. Pp31-35.

Sheldrick, L.E., Flick-Smith, H., Skidmore, J.A., Wensvoort, S., Billah, M., Ali, C. M., and Allen, W.R (1992) LH release profiles in female dromedary camels following mechanical and hormonal stimuli to induce ovulation. In *Proceedings of the First International Camel Conference*. Eds WR Allen, AJ Higgins, IG Mayhew, DH Snow and JF Wade. R&W Publications, New market. Pp 407. Dubai, UAE.

Skidmore, J. A. (2013). Methods to improve the reproductive of camel. In: *Proceedings of the International Scientific Conference of Camel Research and Production (ISCCRP)* 17th-18th April. Khartoum-Sudan. Pp. 83 – 90.

Skidmore, J. A (2011) Reproductive Physiology in Female Old World Camelids. *Journal of Animal Reproduction Science*. **124**: 148–154.

Skidmore, J. A., Billah, M., and Allen, W. R (1996) The ovarian follicular wave pattern and induction of ovulation in the mated and non-mated one humped camel (*Camelus dromedarius*). *Journal of Reproduction and Fertility* **106**:185–92.

SPSS (2015) Statistical Package for Social Sciences. SPSS, Inc., Chicago, IL, USA, Copyright for windows, version 16.0.

Tibary, A., Anouassi, A., Sghiri, A., and Khatir, H (2007) Current Knowledge and Future challenges in Camelid Reproduction. *Society of Reproduction and Fertility*. **64**: 297-313. Nottingham University Press, Nottingham.

Tibary, A., and Anouassi, A (1996) Ultrasonographic changes of the Reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy.

Journal of Camel Practice and Research. **3**: 71–90.

Vyas, S., and Sahani, M. S (2005) Real Time ultrasonography of ovaries and breeding of the one – humped camel (*Camelus dromedarius*) during the early postpartum period. *Journal of Animal Reproduction and Science*. **59**: 179-184.