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RESEARCH ARTICLE

Comparative Efficacy of GnRH, HCG and Seminal Plasma for Induction of Ovulation During the Breeding Season in the Dromedary Camel *(Camelus dromedarius)*

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A B S T R A C T

In the current study, efficacy of GnRH, hCG and seminal plasma for the induction of ovulation in dromedary camels during breeding season was investigated. Sixteen female dromedary camels, having ovarian follicle size of 12-19mm, were randomly divided into four equal groups G, H, SP and C. The camels in groups G and H were injected with 2ml (50µg) GnRH analogue and 3000IU of hCG, respectively. Camels in group SP were injected with 1ml seminal plasma mixed with 1ml normal saline and group C (control group) was injected with 1ml normal saline intramuscularly. For the confirmation of ovulation, and CL development, ovaries of experimental camels were examined through trans-rectal ultrasonography on days 1, 2, 3, 8 and 11 post-treatment. The Progesterone ELISA kit was used to determine serum progesterone (P4) concentrations in samples collected on days 0, 3, 6, 8, and 11 of treatment. The results revealed that ovulation rates were 75, 50, 50 and 0% in groups G, H, SP and C, respectively, values for G, H and SP groups were higher compared to group C ($p<0.05$), while former three groups differed non-significantly from one another. Serum P4 concentrations were <0.5ng/ml in camels of all groups on day 0. However, among ovulated camels, the serum P4 concentration was highest on day 8, when it was 4.80±0.15, 4.15±0.14 and 2.67±0.15ng/ml in SP, H and G groups, respectively; the difference among 3 groups was significant ($p<0.05$). It was concluded that in dromedary females, use of hormones for the induction of ovulations is an effective option and can safely be used in camel breeding programs, as hormones are easily available compared to seminal plasma.

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INTRODUCTION

Camel breeding is gaining popularity in different countries of the world, including Pakistan. Globally, camel population is 26 million heads, out of which 1.1 million camels are present in Pakistan (Faraz *et al.*, 2019). The female camel usually attains puberty at the age of 3 years. However, the recommended breed-able age is 3.5 to 4 years. Male camel attains puberty at the age of 4 years, but full reproductive activity is achieved at the age of 6 to 7 years (Skidmore, 2011).

The estrous cycle in camel occurs in a regular follicular wave pattern (average duration of 24-28 days) during the breeding season (Skidmore and Billah, 2006). Each follicular wave consists of four phases, namely recruitment phase, growth phase, maturation phase and regression phase (Khalifa *et al.,* 2016). The recruitment phase starts with the recruitment of about 8 to 34 follicles (average about 12 follicles). During growth phase 3 to 6 follicles grow under the influence of follicle stimulating hormone (FSH), until they reach 8mm in diameter, while during maturation phase 1 to 2 growing follicles change into dominant follicle and continue to develop into ovulatory (Graafian) follicles with 12 to 18mm in diameter (Manjunatha *et al.,* 2022). If mating occurs during this phase, then ovulation occurs and a corpus luteum (CL) develops (Monaco *et al.,* 2015). In the absence of mating, large anovulatory or regressing follicles can be observed during regression phase and estrous period will be extended (Skidmore, 2011).

The cyclic studies, using real-time ultrasonography and hormone assays to monitor ovarian follicular changes (Vyas *et al.,* 2004) have shown that the follicular wave pattern varies considerably between camels and a mature follicle reaches a size of 13-17mm in diameter in dromedary camels (Skidmore, 2005) and 11.7-25.0mm in Bactrian camels (Nikjou *et al.,* 2009).

Ovulation is triggered through a cascade of neuroendocrine changes that involve signaling pathway between the brain and reproductive organs (Adams and Ratto, 2013). In camelidae, the factors associated with mating are responsible for triggering GnRH and LH secretion that induces ovulation. According to Manjunatha *et al.* (2012) and Anouassi and Tibary (2013), the seminal plasma (SP) contains ovulation inducing factor (OIF), which also contributes to ovulation in females of this species. The OIF is absorbed through genital mucosa, enters into systemic circulation and reaches the adenohypophysis of the female, where it stimulates the LH release that induces ovulation of ovulatory follicle (Yaqoob *et al.*, 2017).

The previous studies have shown that intramuscular administration of SP can induce the preovulatory LH spike, which results in ovulation and subsequent corpus luteum formation in camelids (El Allali *et al.,* 2017). The OIF in the seminal plasma is a neurotrophin, the subunit of nerve growth factor (NGF), and is well known for increasing neuron survival and proliferation. It also appears to trigger ovulation via an endocrine mode of action (Adams *et al.,* 2005). In fact, NGF may enter the body through the endometrium and travel through the circulation to the tissues controlling the preovulatory LH surge (Kumar *et al.,* 2013).

The control of ovulation is of fundamental importance in animal breeding programs and in camelidae ovulation can be induced by depositing seminal plasma deep into the vagina or by intramuscular, subcutaneous, or epidural administration of GnRH, hCG or LH (Monaco *et al.,* 2017). Moreover, ovulation can also be induced by mating with sterile male camel, but this is prohibited due to risk for the spread of venereal diseases. Physical manipulation of cervix, intrauterine deposition of distilled water and cloprostenol does not release sufficient amount of GnRH and LH from hypothalamus and adenohypophysis respectively, to induce ovulation (Ahmed and Mustafa, 2016). According to Monaco *et al.* (2015), manual stimulation of the cervix for 15 minutes only caused partial luteinization of dominant follicle without ovulation.

In the camel, occurrence of ovulation also depends upon the diameter of the dominant follicle. Ovulation rate is up to 85% if size of dominant follicles ranges from 12 to 19mm (Mohamed *et al.,* 2021). The ovulation rate decreases to 12.5% if the size of dominant follicles is between 20 and 29mm, while there is no ovulation if dominant follicles have diameter greater than 30mm. Although the follicles that are 10mm in diameter can be ovulated, but the functionality of developed CL will be impaired (Ghallab *et al.,* 2022). Therefore, the diameter of dominant follicle should be between 12 and 19mm for successful induction of ovulation. In the dromedary camels, ovulation of dominant follicle mostly occurs 24 to 36 hours post-administration of GnRH or hCG hormone (Purohit *et al.,* 2020). Moreover, ovulation can be induced within 30 to 48 hours post-administration of seminal plasma I/M or S/C. However, if trypsin protein is present in the seminal plasma, ovulation will not occur. Under the influence of LH, ovulation takes place 32-40 hours after natural mating (Ali *et al.*, 2010).

Like other mammals, CL is the main source of progesterone (P4) hormone in she camel. Without mating and subsequent ovulation, the plasma P4 concentration remains less than 1ng/ml throughout the breeding season (Bekkouche *et al.,* 2022). The plasma P4 concentration remains low for the first three to four days after ovulation, then increases upto 3ng/ml on day 8, again starts to decrease from day 9 and then reasches 1ng/ml on day 11 to 12 (Khalifa *et al.,* 2020). The use of reproductive biotechnology with novel ovulation induction methods in camels is badly needed to explore the genetic potentials of this species.

Therefore, the present project was designed to evaluate the comparative efficacy of ovulation induction methods in terms of ovulation rates, CL size and serum progesterone concentrations in Dromedary females in order to add some information in the existing knowledge about camel reproduction.

MATERIALS AND METHODS

Research site and selection of experimental animals: The current research was conducted during the months of January to March 2020 at the Camel Breeding and Research Station Rakh Mahni, District Bhakkar, Punjab, Pakistan. A total of 16 adult, healthy, cyclic female camels (*Camelus dromedarius*), aged 6-10 years and having ovarian follicles of 12-19mm diameter were selected from the camel herd during breeding season, which in Pakistan normally extends from December to March. Before selection, follicle size on ovaries was measured through trans-rectal ultrasonography by using a real time, B-mode ultrasound scanner fitted with a linear array transducer of 7.5 MHz frequency (Fig. 1). For this purpose, female camels were restrained in sternal recumbence, size of each ovarian follicle was measured twice, and the mean value was calculated. Females with follicle size 12-19mm were selected for the study (Mohamed *et al.,* 2021). All the selected animals were kept under similar housing and management conditions i.e., under semi-intensive farming, where they were fed on grains, wheat straw, natural grazing and provided clean water ad-libitum.

These camels were randomly divided into four experimental groups i.e., G, H, SP and C, with four camels in each group. The camels in groups G and H were injected with 2ml (50µg) of GnRH analogue (Dalmeraline®, Lecirelin acetate, Fatro) and 3000IU of hCG (Ferti C®, RG Pharmaceutical), respectively. Camels in group SP were injected with 1ml seminal plasma mixed with 1ml normal saline solution intramuscularly. Group C served as control group and was injected with 1ml normal saline.

Determination of ovulation and CL development: The ovaries of experimental female camels were examined through trans-rectal ultrasonography on days 1, 2, 3, 8 and 11 post-treatments to assess the occurrence of ovulation and corpus luteum (CL) development in terms of its size.

Determination of serum P4 concentrations: For serum collection, blood samples were collected from each animal on day 0 (pre-treatment) and days 3, 6, 8 and 11 post-treatments. Serum was separated and the labeled Eppendorf tubes containing serum were stored in a deep freeze at -20ºC. Serum P4 concentrations were measured by using commercially available Progesterone Enzyme Immunoassay Test Kit (BioCheck, EIA Kit, Inc, Foster City, CA 94404, Catalogue No 200048). The sensitivity of BioCheck progesterone EIA assay was 0.0625ng/ml. The inter-assay CV was <12% and the intra-assay CV was <10%.

Statistical analysis: The data thus obtained were subjected to Chi-square test and Analysis of Variance (ANOVA) with Minitab® statistical software. The data for follicle size at day 0 was analyzed through one-way ANOVA, while data on CL size and serum P4 concentrations at various days were analyzed by using two-way ANOVA, followed by Duncan's multiple range test where the main effect was significant. The data on ovulation rate was analyzed by the Chi square test. The results were considered significant at $p<0.05$ level.

RESULTS

Ovulation rates and follicle size: The results regarding ovulation rate and follicle size are depicted in Table 1. The ovulation rates were 75(3/4), 50(2/4), 50(2/4) and 0(0/4)% in females of groups G, H, SP and C, respectively. The ovulation rates for G, H and SP groups were significantly higher compared to that of group C ($p<0.05$), while former three groups differed non-significantly from one another. In group G, two females ovulated after 48 hours and one after 72 hours of treatment. In group H, both females ovulated after 48 hours of treatment. In group SP, one female ovulated after 48 hours and one after 72 hours of treatment.

Mean (±SD) follicle diameter was the highest in female camels of group H (16.65±2.60mm) and lowest was 14.67±1.08mm in females of control group C. Statistical analysis revealed that mean follicle diameter was significantly higher in females of groups H, G and SP compared to control group. However, the difference in follicle diameter among females of H, G and SP were non-significant (Table 1).

Corpus luteum development: The corpus luteum (CL) development during different days of luteal phase is shown in Table 2. In camels of all three treatment groups (G, H & SP), the size of CL showed a significant increase ($p<0.05$) from Day 3 to Day 8, followed by a significant decrease on Day 11. This shows that development of CL was significantly affected by days after treatment, being highest on Day 8 after treatment in all treatment groups. However, the differences in size of CL among camels of three groups i.e., G, H and SP on days 3, 8 or 11 were non-significant. This indicates that three treatments had no effect on the development of corpus luteum.

Serum progesterone concentrations: A significant effect ($p<0.05$) of treatments on serum P4 concentrations in ovulated female camels was recorded on days 6 and 8 only (Table 3). On day 0, 3 and 11 post-treatment, a non-significant difference in the serum P4 concentrations was observed among ovulated females of groups G, H and SP (Table 3). However, on day 6, the serum P4 concentration was significantly higher ($p<0.05$) in the female camels of group SP as compared to those of groups G and H, the difference between the latter two groups was non-significant. On day 8, serum P4 concentration was the highest in females of SP group, followed by those of H group, while the lowest value was found in camels of group G, the difference among three groups was statistically significant ($p<0.05$). The interaction between treatments and days for serum P4 concentration was also significant ($p<0.05$).

In all ovulated groups (G, H & SP), serum progesterone concentrations increased from day 0 to Day 8, followed by a decrease on Day 11 of the study ($p<0.05$). Among non-ovulated female camels, serum P4 concentrations on different days pre- and post-treatment (0, 3, 6, 8, and 11) remained less than 0.5ng/ml, with non-significant differences among all groups throughout the research trial, as shown in Table 3.

DISCUSSION

For successful reproduction, ovulation is of prime significance to establish pregnancy. The camelidae are induced ovulatory species (Skidmore, 2011) which usually ovulate 24-48 hours after mating, use of ovulatory hormones or other ovulatory stimuli. In the camel, estrus cycle lasts for about 28 days; after ovulation CL maintains its size for 13 days before regressing in 08 days. In most animals, ovulation is triggered through a cascade of neuroendocrine changes that involve signaling pathway between the brain and the reproductive organs (Silva *et al.,* 2012). Ovulation is a complicated mechanism initiated by pre-ovulatory LH surge and controlled by many genes, growth factors and estrogen receptors (Skidmore, 2011). For ovulation, preovulatory LH surge causes many biochemical changes in the ovulatory follicle such as release of histamine (Adel *et al.,* 2020), production of prostaglandin (Rateb *et al.,* 2020), enzymatic degeneration, and contraction of follicular smooth muscles that lead to ovulation (Robker *et al.*, 2018).

The induction of ovulation in camels has been reported by 1/M injection of seminal plasma (Duggal *et al.*, 2002), GnRH (Carluccio *et al.,* 2007), LH/hCG (Brännström and Enskog, 2002), and growth hormone-releasing hormone (Adams and Ratto, 2013), with variable results. In the current research, ovulation in female camels was induced by I/M injections of GnRH, hCG and seminal plasma and the ovulation rates were 75, 50 and 50%, respectively compared to 0% in control group. The differences in ovulation rates for GnRH, hCG and seminal plasma were non-significant, indicating that GnRH, hCG and seminal plasma are equally effective for the induction of ovulation in Dromedary females. Such findings highlight the role and significance of GnRH, hCG and seminal plasma in the reproduction of camels. These findings are supported by studies in which single injection of GnRH and hCG caused ovulation in Llamas (Adams and Ratto, 2013).

It was also observed that female camels which ovulated on day 2nd of treatment had greater than 19mm CL on their ovaries, whereas camels that ovulated on day 3 of treatment had smaller than 19mm CL on their ovaries on day 8.

Table 1: The follicle size (mean±SD) and ovulation rate in Dromedary camels of four study groups.

Study groups	Follicle size (mm)	No. of female camels	No. of ovulated female camels	Ovulation rate (%)
G	15.70 ±1.99^{ab}	4	3	75^{a}
H	16.65±2.60^{a}	4	2	50^{a}
SP	15.17±0.85^{b}	4	2	50^{a}
C	14.67±1.08^{c}	4	0	0^{b}

Values having different superscripts in a column show significant difference from one another ($p<0.05$).

Table 2: Mean (±SD) values for the size of corpus luteum during luteal phase in ovulated study groups on days 3, 8 and 11 post-treatments.

Days post treatment	No. of ovulated female camels	Study groups		
		G	H	SP
3	3	16±1.21^{Ab}	17±0.00^{Ab}	15±2.10^{Ab}
8	2	20±2.00^{Aa}	20±0.00^{Aa}	18.5±2.12^{Aa}
11	2	10.67±1.15^{Ac}	12.5±0.71^{Ac}	10.0±0.00^{Ac}

Mean (±SD) values having different lower-case superscripts in a column or upper-case superscripts in a row show significant difference from one another ($p<0.05$).

Table 3: Mean (±SD) serum P4 concentrations (ng/ml) in ovulated and non-ovulated female camels at different days (pre- and post-treatments).

Study groups	Day 0	Day 3	Day 6	Day 8	Day 11
			Ovulated female camels		
G	0.44±0.34^{g}	1.21±0.1^{de}	1.55±0.57^{d}	2.67±0.15^{c}	0.88±0.5^{ef}
H	0.49±0.44^{gf}	1.3±0.28^{de}	1.55±0.14^{d}	4.15±0.14^{b}	1.03±0.99^{e}
SP	0.50±0.1^{gf}	1.23±0.21^{de}	2.10±0.14^{c}	4.80±0.15^{a}	0.84±0.21^{ef}
			Non-ovulated female camels		
G	0.47±0.00^{a}	0.54±0.00^{a}	0.56±0.00^{a}	0.49±0.00^{a}	0.53±0.00^{a}
H	0.46±0.08^{a}	0.42±0.00^{a}	0.44±0.28^{a}	0.40±0.14^{a}	0.40±0.02^{a}
SP	0.470.01^{a}	0.38±0.07^{a}	0.42±0.06^{a}	0.43±0.00^{a}	0.42±0.05^{a}
C	0.41±0.05^{a}	0.43±0.18^{a}	0.42±0.04^{a}	0.40±0.18^{a}	0.38±0.02^{a}

Mean (±SD) values having different superscripts in a column show significant difference ($p<0.05$).

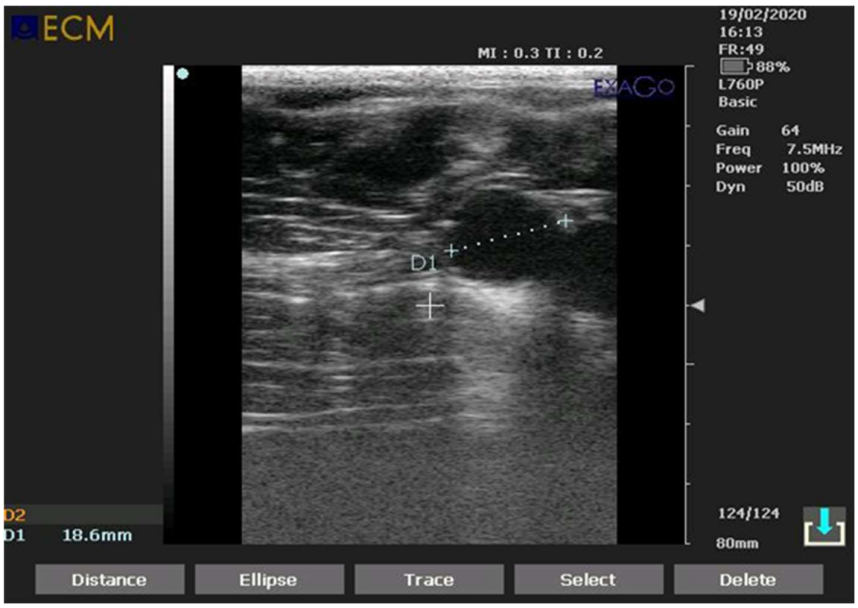

Fig. 1: Ultrasonograph showing follicle size measurement on the ovary of a female camel through B-mode, trans-rectal ultrasonography. The size of measured follicle is 18.6mm.

Similar results have been reported in earlier studies, which showed the development of CL and ovulation in female camels occurred within 24 to 48 hours post administration of GnRH or its analog Buserelin through intramuscular or intravenous route (Nagy *et al.,* 2005; Adams and Ratto, 2013). On the other hand, Vyas and Sahani (2000) reported that ovulation in dromedary female camels did not occur up to 96 hours post Buserelin administration. Similarly, in current study, the female camels having ovarian follicle diameter greater than 15mm ovulated on day 2 and the female camels with ovarian follicle less than 15mm in diameter ovulated on day 3 of treatment.

In the current study, serum P4 concentration was below 0.5ng/ml pre-treatment in all female camels. Previous studies also showed that serum progesterone concentrations remained less than 1ng/ml throughout the estrus cycle without succeeding ovulation (Nagy *et al.,* 2005; Skidmore and Billah, 2006). However, serum P4 concentrations increased in female camels that ovulated following administration of GnRH, hCG or seminal plasma. This increase in serum P4 concentration was observed on days 3, 6 and 8, followed by a decrease on day 11. The seminal plasma increased the serum P4 concentration more as compared to hCG and GnRH. Basically, seminal plasma increased the size of CL up to 18.5mm and functions which ultimately enhanced the production of serum P4 (Ali *et al.,* 2010; Bekkouche *et al.*, 2022).

In non-ovulated female camels, serum P4 concentrations remained less than 1ng/ml throughout the study. The results of statistical analysis for the effect of treatments on serum progesterone concentrations in non-ovulated female camels indicated that treatments had no

effect on serum progesterone concentration in non-ovulated females. According to Nagy *et al.* (2005) and Skidmore (2011), serum progesterone concentrations remained less than 0.5ng/ml in non-ovulated female camels. The number of animals in each study group was 4 due to the availability of a smaller number of animals during the breeding season. However, this number of animals in each study group worked for an appropriate statistical design to draw some conclusion.

Conclusions: In dromedary females, ovulation can be induced by intramuscular administration of GnRH, hCG or seminal plasma, with non-significant differences in ovulation rates, although GnRH treated female camels had 25% higher ovulation rate. In general, serum P4 concentrations were higher in seminal plasma treated she-camels compared to GnRH or hCG treated animals.

Ethical approval: An ethical approval of study protocol was taken from the Institutional Biosafety and Bioethics Committee (IBC), University of Agriculture Faisalabad, Pakistan to conduct this study.

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Authors contribution: ZIQ and MSW conceived the idea and designed this study. DA executed the experiment and collected samples. ZIQ, MSW and IH provided the technical facility for smooth execution of research. DA, MI and MWU analyzed the data and drafted the manuscript. All authors interpreted the data, reviewed the manuscript, and approved the final version.

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