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# Singular epidural follicle-stimulating hormone administration on follicular growth in camels

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## Abstract

Super-stimulation enables to obtain multiple oocytes and/or embryos but requires laborious and stressful multiple hormonal injections. To simplify the super-stimulation process, we evaluated the effect of a single epidural follicle-stimulating hormone (FSH) administration in camels. After the epidural treatment, 71% of camels showed multiple ovulatory follicles, the number of ovulatory follicles were lower after the epidural treatment (mean  $\pm$  standard deviation,  $6.0 \pm 6.7$ ) than the conventional treatment ( $22.4 \pm 13.9$ ), which consisted of a single injection of equine chorionic gonadotropin (eCG) and daily FSH injections for 8 days, and was similar to eCG alone ( $11.4 \pm 13.7$ ). In conclusion, singular epidural FSH administration was not comparable with the conventional protocol but still successfully induced super-stimulation in camels.

Key Words: camel, epidural administration, follicle-stimulating hormone (FSH)

Camel (*Camelus dromedarius*) reproduction and cloning have received renewed interest lately. Assisted methods for camel reproduction were pioneered in the early 90's and more advanced embryo technology including cloning followed in the following decades<sup>13,18,23</sup>. As the ability to retrieve multiple oocytes and/or embryos is crucial to the success of reproductive technologies; methods of super-stimulation, by treatment with follicle-stimulating hormone (FSH), have long been used to induce follicular growth and improve

oocyte and embryo production in camels<sup>1</sup>. In addition, a combination of equine chorionic gonadotropin (eCG) and multiple FSH injections are widely used, because the combination showed higher embryo productivity than FSH alone<sup>14</sup>. However, conventional super-stimulation consists of multiple intramuscular injections spanning a week and is stressful for the animals and time-consuming for veterinarians. Success additionally depends on the rigors of the execution of individual protocols with some groups obtaining

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varied results<sup>22)</sup>.

Previous attempts in cattle have aimed at simplifying super-stimulation protocols including reducing injection number, with varying results<sup>4,6,9,20)</sup>; similar studies have been reported in camels<sup>2,12)</sup>. Some reports have included the use of various molecules to slow the release of FSH from a single injection<sup>9,20)</sup>.

In cattle, a single epidural FSH administration, via the caudal vertebrae, has been reported to be as effective in the induction of super-stimulation as a comparable conventional multiple FSH injection protocol<sup>16)</sup>. In humans, following epidural administration, anesthetic drugs are absorbed into epidural fat, then gradually absorbed into the general circulation<sup>5)</sup>. In cattle, epidural fats are thought to delay the movement of FSH into the peripheral circulation, and FSH concentration can be maintained for more than 3 days at higher than basal levels<sup>16)</sup>. Epidurals are routinely administered in a number of species including camels, primarily with anesthetic drugs for reproductive work, and are considered to be a safe and effective method of drug delivery<sup>8)</sup>. Taken together, epidural injection of FSH is possible to induce super-stimulation even in camels. The purpose of this study was to confirm whether super-stimulation could be induced by a single epidural administration and evaluate the efficiency of super-stimulation by a single epidural FSH administration, in camels. It has previously been reported that a single intramuscular administration of eCG induced super-stimulation, in camels, while the number of embryos collected from eCG-treatment were found to be fewer than that from camels treated by twice daily administration of FSH for 3 to 5 days<sup>21)</sup>. In addition, the combination of a single eCG administration and multiple FSH administrations is known to show better stimulation than eCG or FSH alone<sup>21)</sup>. Therefore, we compared the growth of ovulatory follicles in a single epidural administration with a single eCG administration and the combination of a single eCG administration and multiple FSH

administration.

We randomly selected female camels which had a normal breeding history and the absence of abnormalities in the reproductive tract based on an ultrasonic examination. Animals were fed appropriate nutrients and provided water *ad libitum* daily. All animal procedures were conducted following the animal study guidelines, which were approved by the ethics committee at the Management of Scientific Centers and Presidential Camels (Accession No: PC4.1.5).

All camels also received 500 µg of cloprostenol (Jurox, Rutherford, Australia) at random stage of follicular growth during a reproductive season (day 0). To evaluate the effect of a single epidural FSH injection on *in vivo* follicle production, camels were divided into three groups (conventional, eCG, and epidural). The conventional group (n=7) were given intramuscular pregnant mare serum gonadotropin (Syncropart 6000 IU, Lyophilized serum gonadotrophin, Ceva, Libourne, France) on day 0 and intramuscular FSH (Follitrophin-V, 400 mg NIH, Vetoquinol, Paris, France) dissolved in 26 ml normal saline was administration for 8 days with the following protocol: 3 ml twice on the first and second day followed by 2 ml twice daily for the next two days, then 1 ml twice daily for two days and a single 1 ml injection in the morning of the final two days. Although super-stimulation is reported to be induced by twice daily injection of porcine FSH for 3 days in camel<sup>13)</sup>, which is same duration as cattle, our conventional protocol is 8 days considering that camels has longer follicular growth phase ( $10.5 \pm 0.5$  days) and maturation phase ( $7.6 \pm 0.8$  days) than cattle (mean  $\pm$  standard error of the mean)<sup>17)</sup>. The eCG group (n=14) received only a single intramuscular injection of 6000 IU eCG on day 0 without the addition of FSH. The epidural group (n=7) was administered FSH (400 mg) dissolved in 5 ml of saline, by epidural injection in the sacrococcygeal or the first or second coccygeal intervertebral space on day 0. The total amount of FSH (400 mg) in the epidural group was same as the total amount of the conventional group.

**Table 1.** Super-stimulation response induced by conventional treatment using follicle-stimulating hormone (FSH) and equine chorionic gonadotropin (eCG) compared with a single epidural administration of FSH, or a single intramuscular administration of eCG

Treatment (replicates)	No. of follicles at estrus ( $\geq 10$ mm)	Camels with multiple follicular growth (%)	Camels with cystic follicles (%)
Conventional (n=7)	22.4 $\pm$ 13.9 <sup>a</sup>	100	43
Epidural (n=7)	6.0 $\pm$ 6.7 <sup>b</sup>	71	43
eCG (n=14)	11.4 $\pm$ 13.7 <sup>ab</sup>	79	36

<sup>a, b</sup> Different letters indicate significant differences between each group ( $P < 0.05$ ).

No. of follicles at estrus is described as mean  $\pm$  standard deviation.

Groups were treated simultaneously to minimize intergroup variation. On day 9, when ovulatory follicles are observed and the appropriate timing to induce ovulation<sup>15,19</sup>, the number of large follicles ( $\geq 10$  mm in diameter) was counted using ultrasonography and compared between groups. The presence of multiple follicular growth was defined by 2 or more follicles present in a pair of ovaries. Follicles larger than 30 mm in diameter were designated as cystic follicles. The percentage of camels with multiple follicular growth and the percentage of camels with cystic follicles were compared between the groups. Untreated camel ovaries were obtained from a local abattoir to determine a baseline for the presence of cystic follicles.

All statistical analyses were performed using software (JMP Pro 14, SAS Institute, Cary, NC, USA). The normality of distribution of the number of large follicles in each group was analyzed by the Shapiro-Wilk W test. As some data did not show normal distribution, the number of large follicles was analyzed by non-parametric the Kruskal-Wallis test followed by the Steel-Dwass test. Data described as percentages were analyzed by the Fisher's exact test.

As shown in Table 1, the number of large follicles ( $\geq 10$  mm in diameter) obtained was significantly higher in the conventional group

than in the epidural group ( $P = 0.008$ ). The epidural group was roughly equivalent to eCG treatment alone. In the conventional group, multiple follicular growth was successfully induced in all camels, compared to the epidural (71%) and eCG (79%) groups. The percentages of animals exhibiting induced multiple follicular growth was statically similar among the epidural and eCG groups ( $P = 0.5463$ ). The percentages of camels with cystic follicles were similar among the groups (36-43%,  $P = 1.000$ ). Non-treated ovaries were observed to have cysts at the same approximate rate of 19% of ovaries (n=42), or approximately 38% of camels.

In the present study, a single epidural injection of FSH induced multiple follicular growth in 5 of 7 camels (71%), although achieved, efficiency of super-stimulation was not comparable with the 100% response of the conventional regimen, consisting of multiple injections. The biological half-life of FSH is reported to be five hours, injected intramuscularly in cattle<sup>7</sup>. In cattle, the number of transferable embryos collected after a single epidural FSH injection was similar to that following multiple FSH injections for 3 days<sup>16</sup>. Our experiment included a longer period than in the studies in cattle, following the conventional protocols timeline of 8 days. The reduced number of follicles in the epidural group may indicate that the duration between epidural FSH injection and observation of follicular growth was too long and a degeneration of ovulatory follicles may have occurred. Further studies would be needed to establish a suitable duration between epidural injection. Plasma FSH profiles would be valuable in both this determination and for the optimization of both single and multiple injection protocols.

There was wide variation observed in ovulatory follicle number between individuals, especially in the epidural (mean  $\pm$  standard deviation, 6.0  $\pm$  6.7) and the eCG groups (11.4  $\pm$  13.7). Bo *et al.* reported that the amount of subcutaneous fat affected outcome of superovulation; superovulation could be induced

in cross-bred beef cows with a high body condition score (3 to 5, 1: very thin, 5: very fat); however, superovulation was not induced in cows with a low body condition score<sup>3)</sup>. In addition, the amount of epidural fat was known to affect the distribution of drugs in the epidural area<sup>10,11)</sup>. Wide variation in the number of ovulatory follicles in the epidural group and eCG group may relate to these reported differences.

eCG was not administered along with the single FSH epidural injection in the present study; in order to determine a cumulative effect, a separate epidural administration including an advanced eCG administration may be warranted. This has been shown in the literature by Nowshari *et al.*, who suggested that camels treated with a combination of eCG and multiple FSH injections obtained a higher number of transferable embryos than camels treated with FSH alone<sup>14)</sup>. In light of Nowshari *et al.*'s findings the efficiency of the combination of a single injection of eCG and epidural FSH may also warrant investigation.

The observed presence of cystic follicles prompted a comparison to untreated ovaries from a local slaughterhouse. We found that ovaries with cystic follicles occurred at the same frequency as the observation in the super-stimulation experiments. Approximately 19% of untreated ovaries exhibited cystic follicles, equivalent to approximately 38% of camels. This frequency was within the range of our observation of super-stimulated camels, indicating that the presence of follicular cysts was independent of administrations (Table 1).

In conclusion, although a single epidural administration was not as consistent, nor did it produce a follicle number equivalent to the conventional method and was equivalent to the use of eCG alone, a single epidural administration of FSH successfully induced super-stimulation. Further study towards increasing the effectiveness of this method along with the combination with eCG administration is warranted.

### Conflicts of Interest

None declared.

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