



Effects of Recombinant Follicle Stimulation Hormone on the Ovarian Response in Domestic Cats

Nahid Changalvaei¹, Farid Barati ^{1,*}, Moosa Javdani¹, Katayoon Oskouizadeh¹

¹ Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

*Corresponding Author: Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran. Email: baratif@sku.ac.ir

Received: 7 October, 2025; Revised: 1 November, 2025; Accepted: 4 November, 2025

Abstract

Background: Recombinant follicle-stimulating hormone (rFSH) can be administered for the manipulation of the estrus cycle and controlled ovarian stimulation in domestic cats, serving as a crucial model for endangered felid assisted reproductive technology (ART).

Objectives: The present study aimed to evaluate the effects of different doses and durations of rFSH administration on ovarian volume and oocyte quality in anestrous domestic cat queens.

Methods: A total of 12 anestrous queens were randomly allocated into four groups (n = 3 per group): Three active treatment groups receiving rFSH (Cinnal-F, Iran) intramuscularly and one negative control group (G4, saline). Treatment groups compared dose and duration: G1 (2.0 mg × 2 days), G2 (2.5 mg × 2 days), and G3 (2.5 mg × 4 days). After ovariectomy, ovarian volume was estimated using its dimensions, and oocytes were surgically retrieved 24 hours post-final injection, then counted and graded morphologically (A, B, C/D).

Results: The ovarian volume (mm³) was the highest in the 4.0 mg total dose group (right ovary: 178.7 ± 16.8; P = 0.0237; left ovary: 128.2 ± 17.1; P = 0.0405) among all other groups. However, logistic regression analysis revealed no significant differences among any of the groups (G1 - G4) regarding the odds ratios for retrieving high-quality oocytes (grades A and B).

Conclusions: The findings showed no efficacy of the applied protocol of rFSH on the ovarian response in terms of oocyte retrieval and follicle development.

Keywords: Recombinant Follicle-Stimulating Hormone (rFSH), Ovarian Response, Oocyte Quality, Domestic Cats

1. Background

Recombinant follicle-stimulating hormone (rFSH) remains a vital therapeutic agent in reproductive medicine, offering a highly pure and consistent formulation for infertility treatment. Most clinical research has focused on optimizing its use in assisted reproductive technology (ART), primarily in vitro fertilization (IVF), where its main application is controlled ovarian stimulation to induce the development of multiple follicles (1, 2). Studies continue to confirm the high efficacy and safety of rFSH preparations (follicotropin alfa and beta) in achieving high oocyte yield and favorable clinical pregnancy rates, even when compared against biosimilar or urinary-

derived alternatives (2, 3). A key area of recent investigation involves individualizing treatment, with growing evidence suggesting that adding rFSH may benefit specific patient groups, such as women of advanced maternal age, by significantly increasing live birth rates (4, 5). Furthermore, rFSH was efficient in the super-stimulation of the ovary and embryo recovery in dairy cattle, too (6).

Estrous cycle irregularities are a significant challenge across the Felidae family, impacting both companion animal management and endangered species conservation. In the domestic queen, common problems involve abnormal cyclicity, such as prolonged estrus, which is sometimes linked to cystic ovarian follicles, and ovarian remnant syndrome, where signs of

heat behavior unexpectedly recur in a spayed cat due to residual functional ovarian tissue (7, 8). More broadly across the Felidae, a major hindrance to captive breeding is ovarian quiescence or anestrus, where the female fails to cycle altogether; this condition is often linked to the stress of the captive environment, which can increase glucocorticoid levels and negatively affect ovarian function (9). Furthermore, while all felids are induced ovulators, the frequency of spontaneous ovulation varies widely between species, creating management difficulties for assisted reproduction techniques like artificial insemination in certain wild cats, such as the clouded leopard (10). Therefore, non-invasive techniques like fecal hormone monitoring and accelerometry are increasingly studied to better characterize and manage these complex estrous variations in both domestic and wild felids (9, 10).

The application of FSH in Felidae is fundamental to ART, serving primarily to induce multi-follicular development for the collection of oocytes (gamete rescue) and subsequent use in IVF or AI, particularly for endangered wild felids (10). Current research using the domestic cat as a model has demonstrated that serial exogenous FSH administration effectively reverses the negative effects of the non-breeding season. For instance, treatment of anestrus queens with porcine FSH was found to improve oocyte quality and enhance in vitro developmental competence, resulting in higher rates of blastocyst formation (11). The goal of FSH treatment is to overcome the typical induced-ovulation cycle by promoting the synchronous maturation of multiple follicles (12, 13). However, high variability in individual and species-specific ovarian response to exogenous FSH remains a significant challenge for predictable ART success across the broader Felidae family (10).

2. Objectives

The present study aimed to explore the efficacy of rFSH on ovarian response during early seasonal estrus in domestic cats in Iran.

3. Methods

All experimental procedures involving the domestic cat (*Felis catus*) were conducted in strict accordance with institutional and national guidelines for animal care and use. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Shahrekord University (IR.SKU.REC.1402.045). All animals were housed under standardized conditions

with a natural daily light cycle of 14 hours light/10 hours dark and were provided fresh water and a commercial processed diet (Fidar, Iran) ad libitum.

A total of 12 reproductively intact adult domestic queens, aged 1 - 5 years and weighing 2.5 - 3 kg, were selected during the early seasonally active period (October 2022 - February 2023). Cats were randomly allocated into four experimental groups (n = 3 cats per group); groups 1 and 2 were cats that received daily 2.0 and 2.5 mg rFSH for 2 consecutive days, respectively. Group 3 comprised cats that received daily 2.5 mg rFSH for four consecutive days. Group 4 was designed as a control receiving normal saline. The treatment consisted solely of rFSH (Cinnal-F, Cinnagen, Iran). All injections were administered intramuscularly at approximately 08:00 - 10:00 hours daily.

All cats were examined for ovaries to confirm the lack of corpus luteum on their ovary at the beginning of the study using a 10 MHz abdominal transducer (Mindray, China). The ovary volume was measured using the dimensions (length, width, and height) of both ovaries. Ovarian volume was calculated using the formula for a prolate spheroid: The total ovarian volume for each cat was recorded as the left and right ovarian volumes. Volume was estimated based on the formula: $V = (4/3)\pi a^2c$; where $a = W/2$ and $c = L/2$, W = mean radii of width and height, and L = radius of length.

Ovariectomy was performed surgically 24 hours after the final rFSH. Ovaries were immediately transported to the laboratory in warm phosphate-buffered saline (PBS) supplemented with 1000 IU/L penicillin (Denazist Asia Co., Iran) and 1 mg/L streptomycin (Denazist Asia Co., Iran). Oocytes were recovered by surgically slicing the ovarian cortical tissue into small fragments and flushing the fragments with the previous medium supplemented with 5% fetal bovine serum (Zistpajouh Co., Iran) and 100 IU/L of sodium heparin sulfate (10000 IU/mL; Alborzdaru, Iran).

All recovered oocytes [cumulus-oocyte complexes (COCs)] were counted under a stereomicroscope to determine the total number of retrieved oocytes per cat. The oocytes were categorized based on cumulus investment and morphological integrity into grades A, B, and pooled C and D. Briefly, COCs with more than 3 granulosa layers and homogeneous cytoplasm were considered as grade A. The COCs with less than one and two layers of granulosa layers were grade B. Denuded recovered oocytes with homogeneous cytoplasm were considered as C, and decomposed cytoplasm were categorized as D.

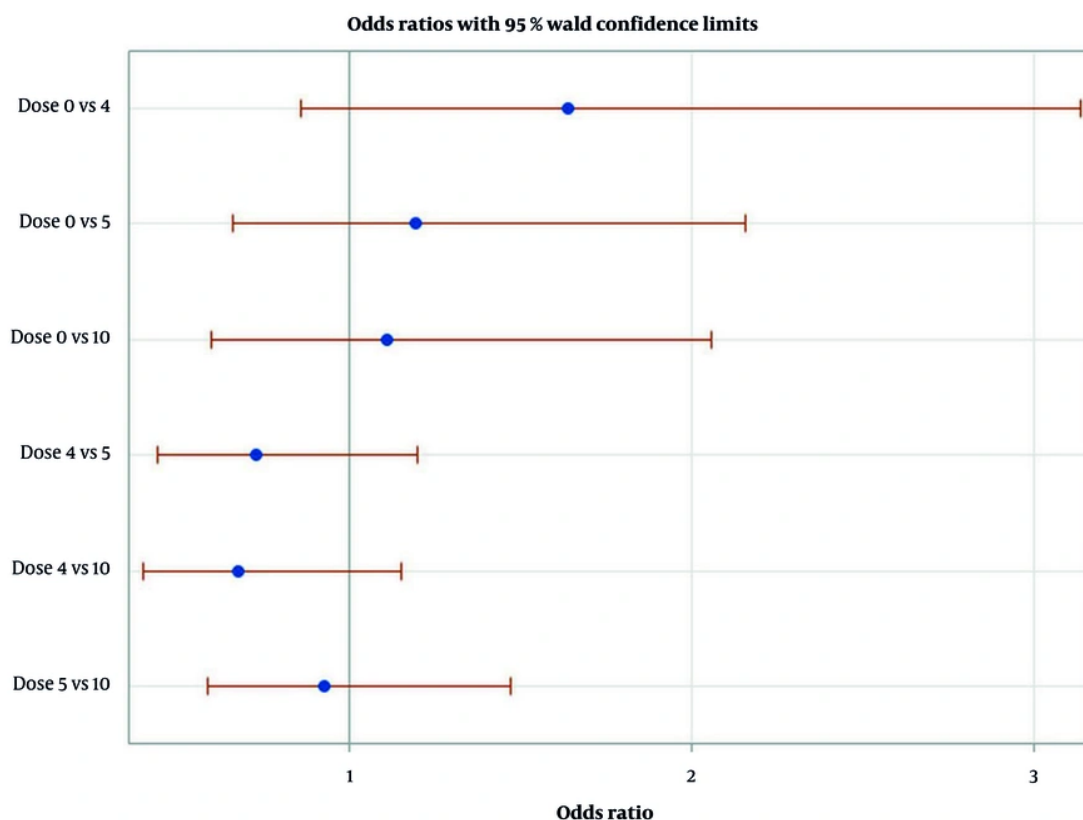


Figure 1. Estimated odds ratios with a 95% confidence interval of good quality oocytes (grades A and B; n = 279) to low quality oocytes (grades C and D; n = 191) compared between different doses

3.1. Statistical Analysis

Logistic regression was used to analyze the effects of treatments on the quality of retrieved oocytes. Ovarian volume was compared among groups using the Kruskal-Wallis test. Results are presented as the mean $\pm$ standard error of the mean (SEM), with significance set at $P < 0.05$. The data were analyzed using the Statistical Analysis System (SAS) 9.4.2.

4. Results

A total of 470 oocytes were recovered from the ovaries of all cats, with 194 (41.3%), 85 (18.1%), and 191 (40.6%) being qualified as grades A, B, and C/D, respectively. By pooled data, the total oocytes per ovary were 10.7 ± 6.19 ($n = 3$) for cats that did not receive rFSH and 19.6 ± 3.2 ($n = 12$) for cats that received rFSH ($P =$

0.1989). Table 1 shows the effects of different treatments on ovary volume and the number of retrieved oocytes. Based on Table 1, ovary volume was significantly higher in group 1 compared to other groups. Logistic regression analysis (Figure 1) shows no difference among groups for the odds ratios of good quality oocytes (grade A+B) to low quality oocytes (C+D).

5. Discussion

Our results showed no beneficial effects of rFSH on follicle development and oocyte retrieval in Iranian domestic cats. Comparative studies on feline ovarian stimulation often focus on optimizing the type and total dose of gonadotropin to move the anestrus queen into the follicular phase. Historically, many protocols relied on pituitary-derived porcine follicle-stimulating hormone (pFSH), which yielded variable results due to

Table 1. The Effects of Different Doses of Recombinant Follicle-Stimulating Hormone on the Ovarian Response of Domestic Cats ^a

Variables	rFSH (means)				SEM	P-Value ^b
	Control	4 mg	5 mg	10 mg		
Ovary volume (mm ³)						
Right	60.2 ^A	178.7 ^B	69.4 ^A	114.6 ^A	16.8	0.0237
Left	40 ^B	128.2 ^A	80.9 ^{A, B}	106.05 ^A	17.1	0.0405
Oocyte per ovary (No.) ^c						
Grade A	5.2 (31)	5.5 (81)	13.5 (51)	8.5 (31)	3.1	0.3243
Grade B	1.7 (10)	2.7 (25)	4.2 (35)	5.2 (19)	1.14	0.2515
Grade CD	3.8 (23)	9.3 (71)	3 (51)	8.5 (46)	2.84	0.2542
Total	10.7	17.5	18.7	22.3	6.07	0.4037
Proportion of oocytes						
Grade A	0.41	0.3	0.5	0.4	0.06	0.2921
Grade B	0.2	0.16	0.16	0.25	0.06	0.5506
Grade CD	0.38	0.53	0.34	0.36	0.09	0.2699

Abbreviations: rFSH, recombinant follicle-stimulating hormone; SEM, standard error of means.

^a Same capital letters indicate not significantly difference ($P > 0.05$).

^b Kruskal-Wallis multiple comparison test.

^c Data in parenthesis are absolute values of oocytes within each group.

its inconsistent purity. The shift to rFSH was aimed at providing consistent biological activity and reducing the risk of immune reactions (11).

The ovarian estimated volume was the highest in the 4 mg group among all, which can be considered as a status of follicular development. Achieving maximal follicular recruitment requires hitting a specific concentration threshold, but increasing the dose beyond this point does not necessarily improve the developmental competence of the retrieved oocytes; instead, it may lead to the retrieval of more atretic or morphologically poor-quality oocytes (13). Our findings suggest that all the active treatment groups (G1 through G3) might have exceeded this minimum threshold, effectively placing them on a response plateau, where further dose increases (e.g., from 4 mg total to 10 mg total) yield statistically similar results in terms of overall ovarian volume and raw oocyte counts.

A critical point of comparison is the duration of treatment, which relates directly to follicular synchronization. The goal of using rFSH is to recruit and synchronize a cohort of follicles that are all ready for final maturation at the time of retrieval. Protocols using shorter durations (e.g., 2 days, like G1 and G2) rely on the high FSH dose overcoming the natural follicular attrition cycle quickly. Conversely, longer protocols (e.g., 4 days, like G3) mimic a more gradual, physiological increase in FSH, theoretically leading to a more evenly

developed group of follicles (10). However, published outcomes show that longer durations can increase the risk of cystic follicular development or premature luteinization, which dramatically reduces the yield of viable, fertilizable oocytes, potentially offsetting any benefit gained from the higher total dose (13). Therefore, if both the short and long protocols resulted in some degree of suboptimal synchronization or hyperstimulation-related issues, the final measured outcomes might indeed converge, masking the intended difference between the 2-day and 4-day regimes.

The intrinsic biological variability frequently overrides subtle differences in hormonal protocols. Published data consistently highlight that the success of ovarian stimulation is highly dependent on the donor cat's inherent physiological state, including age, body condition score, and most importantly, the depth of anestrus at the time of the first rFSH injection (10). Even within a strictly controlled cohort of domestic cats, the number of resting primordial follicles available for recruitment, the individual cat's sensitivity to exogenous rFSH, and the timing of the initiation of the protocol relative to the natural follicular wave all introduce noise. If there was slight variation in the reproductive history or baseline health of the animals within the groups, this physiological variability could easily introduce enough scatter in the final outcome data (ovary volume and oocyte count) to prevent the

relatively small, incremental differences in rFSH doses from reaching statistical significance (11).

On the other hand, the variability in FSH receptor genes and downstream molecular pathways affecting follicular growth in felids may contribute to explaining variable ovarian responses to FSH (14). The results of the present study showed no effects of the administered doses of rFSH for oocyte retrieval in cats. Further studies with a higher sample size are needed to explore the efficacy of rFSH for ovary management in cats.

Acknowledgements

The authors thank Shahrekord University for funding the study. The staff of the Surgery Department and Animal Husbandry Section at the Veterinary Hospital, Shahrekord University, should be acknowledged for their assistance.

Footnotes

Authors' Contribution: Study concept and design: F. B.; Acquisition of data: N. C. and M. J.; Analysis and interpretation of data: N. C. and F. B.; Drafting of the manuscript: K. O. and F. B.; Critical revision of the manuscript for important intellectual content: M. J.; Statistical analysis: F. B.; Administrative, technical, and material support: K. O. and M. J.; Study supervision: F. B. and M. J.

Conflict of Interests Statement: The authors declare no conflict of interests.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The present study was approved under the ethical approval code of IR.SKU.REC.1402.045.

Funding/Support: This study was funded by Shahrekord University.

References

- Li H, He W, Lin H, Du A, Zhang X, Yang H, et al. Recombinant Human Follicle-Stimulating Hormone in Controlled Ovarian Hyperstimulation with Assisted Reproductive Technology in China: A Cost-Effectiveness Analysis. *Risk Manag Healthc Policy*. 2024;**17**:1387-94. [PubMed ID: 38828103]. [PubMed Central ID: PMC1141568]. <https://doi.org/10.2147/RMHP.S456277>.
- Bergandi L, Canosa S, Carosso AR, Paschero C, Gennarelli G, Silvagno F, et al. Human Recombinant FSH and Its Biosimilars: Clinical Efficacy, Safety, and Cost-Effectiveness in Controlled Ovarian Stimulation for In Vitro Fertilization. *Pharmaceuticals*. 2020;**13**(7). [PubMed ID: 32605133]. [PubMed Central ID: PMC7407829]. <https://doi.org/10.3390/ph13070136>.
- Velthuis E, Hubbard J, Longobardi S, D'Hooghe T. The Frequency of Ovarian Hyperstimulation Syndrome and Thromboembolism with Originator Recombinant Human Follicle-Stimulating Hormone (GONAL-f) for Medically Assisted Reproduction: A Systematic Review. *Adv Ther*. 2020;**37**(12):4831-47. [PubMed ID: 33058045]. [PubMed Central ID: PMC7595967]. <https://doi.org/10.1007/s12325-020-01512-w>.
- Bielfeld AP, Schwarze JE, Verpillat P, Lispi M, Fischer R, Hayward B, et al. Effectiveness of recombinant human FSH: recombinant human LH combination treatment versus recombinant human FSH alone for assisted reproductive technology in women aged 35-40 years. *Reprod Biomed Online*. 2024;**48**(6):103725. [PubMed ID: 38593745]. <https://doi.org/10.1016/j.rbmo.2023.103725>.
- Mahony MC, Hayward B, Mottla GL, Richter KS, Beall S, Ball GD, et al. Recombinant Human Follicle-Stimulating Hormone Alfa Dose Adjustment in US Clinical Practice: An Observational, Retrospective Analysis of a Real-World Electronic Medical Records Database. *Front Endocrinol*. 2021;**12**:742089. [PubMed ID: 34956077]. [PubMed Central ID: PMC8696034]. <https://doi.org/10.3389/fendo.2021.742089>.
- Karimi SMJ, Tabatabaei VS, Barati F, Mamouei M. [Effect of Gonadotropin-Releasing Hormone Treatment on Superovulation Response and Embryo Recovery in Holstein Heifers: A Field Trial at Zagros Milk and Meat Company, Iran]. *Gene Cell Tissue*. 2025;**12**(1):1-8. FA.
- Little S. Feline reproduction: problems and clinical challenges. *J Feline Med Surg*. 2011;**13**(7):508-15. [PubMed ID: 21704900]. [PubMed Central ID: PMC1107981]. <https://doi.org/10.1016/j.jfms.2011.05.008>.
- Holst BS. Feline breeding and pregnancy management: What is normal and when to intervene. *J Feline Med Surg*. 2022;**24**(3):221-31. [PubMed ID: 35209770]. [PubMed Central ID: PMC9099157]. <https://doi.org/10.1177/1098612X221079708>.
- Andrews C. *Stress and reproduction in domestic cats (Felis catus) as a model for endangered felids: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Ph.D.)*. Animal Science Group, Massey University, Palmerston North, New Zealand [Dissertation]. Palmerston North, New Zealand: Massey University; 2021.
- Thongphakdee A, Sukparangsi W, Comizzoli P, Chatdarong K. Reproductive biology and biotechnologies in wild felids. *Theriogenol*. 2020;**150**:360-73. [PubMed ID: 32102745]. <https://doi.org/10.1016/j.theriogenology.2020.02.004>.
- Veraguas D, Gallegos PF, Velasquez AE, Castro FO, Rodriguez-Alvarez L. FSH stimulation of anestrus cats improves oocyte quality and development of parthenogenetic embryos. *Theriogenol*. 2017;**87**:25-35. [PubMed ID: 27616216]. <https://doi.org/10.1016/j.theriogenology.2016.08.008>.
- Barnes J, Vansandt L. Estrous cycle manipulation in cats. *Clin Theriogenol*. 2025;**17**:67-81. <https://doi.org/10.58292/CT.v17.i1649>.
- Swanson WF. Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats. *Theriogenol*. 2006;**66**(1):49-58. [PubMed ID: 16650889]. <https://doi.org/10.1016/j.theriogenology.2006.03.024>.
- Neubauer K, Jewgenow K, Fickel J. Follicle-stimulating hormone receptor in felids: intra- and interspecies variation. *Theriogenol*. 2006;**66**(6-7):1737-42. [PubMed ID: 16472852]. <https://doi.org/10.1016/j.theriogenology.2006.01.031>.