Kafkas Univ Vet Fak Derg
18 (5): 879-884, 2012
DOI:10.9775/kvfd.2012-7050

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Induction of Oestrus with Single or Split eCG Administrations in Turkish Van Cats During the Non-breeding Season

Barış Atalay USLU * Fetih GÜLYÜZ * Ömer UÇAR **

* Division of Reproduction and Artificial Insemination, Faculty of Veterinary Sciences, Yüzüncü Yıl University, TR-65080 Van - TURKEY
** Division of Reproduction and Artificial Insemination, Faculty of Veterinary Sciences, Atatürk University, TR-25240 Erzurum - TURKEY

Makale Kodu (Article Code): KVFD-2012-7050

Summary

Effects of equine chrionic gonadotrophin (eCG) upon reproductive traits of Van cats during the non-breeding season were investigated herein. In the non-breeding season (October), a total of 18 queens were divided equally into three experimental groups, as follows: one ml of 0.9% saline solution, as single *placebo* injection (intramuscularly, i.m.) was given to queens, as control (Group I, n=6), while single (250 IU, i.m.) or split dose of eCG (75 IU/daily for 5 d) was injected in Group II and III (n=6 each), respectively. Data from the rates of oestrus, days of mating, pregnancy, parturition, litter size, survival of kittens and lactation period were recorded. The oestrus rates (100% both) in Groups II and III were significantly (P<0.01) higher than those in controls (33.3±21.1%). Days of mating (9.0±1.0, 4.7±0.7 and 5.8±0.4 d) were significantly (P≤0.01) different between the groups (Group I, II and III, resp.). Pregnancy period (65.8±0.5 d) was significantly (P<0.05) longer in Group III ((65.8±1.0 d) than in Group II (62.4±1.0 d) and controls (62.5±0.5 d). The litter size tended (p=0.062) to be higher in Group II (4.6) than those in Group III (2.6) and controls (3.0). Finally, the lactation period (105.2±2.6 d) was significantly (P<0.05) longer in Group III than those in Group II (94.6±1.7 d) and controls (95.0±5.0 d). Findings suggest that; both single and split eCG injections were equally highly effective for induction of fertile oestrus in endemic Van cats in non-breeding season.

Keywords: *eCG, Oestrus induction, Fertility, Van Cat, Non-breeding season*

Anöstrüs Sezonundaki Van Kedilerinde Tek veya Bölünmüş Doz eCG Uygulamaları ile Östrüslerin Uyarılması

Özet

Sunulan çalışmada, üreme sezonu dışındaki Van kedilerinde equine chrionic gonadotrophin (eCG)'nin reprodüktif parametreler üzerine etkileri araştırıldı. Üreme mevsimi dışında (Ekim), toplam 18 kedi eşit olarak üç deney grubuna ayrıldı. Kontrol grubundaki (Grup I, n=6) dişi kedilere 1 ml %0.9 luk tuzlu su, tek *plasebo* enjeksiyonu (intramüsküler, i.m.) yapılırken, Grup II'dekilere (n=6) tek doz (250 IU, i.m.) ve Grup III'tekilere (n=6) 5 gün süreyle bölünmüş eCG dozları (75 IU/gün, i.m.) uygulandı. Östrüs oranı, çiftleşme günleri, gebelik oranı, doğum oranı, bir batında doğan yavru sayısı, yavruların yaşama oranı ve laktasyon periyodu verileri kaydedildi. Östrüs oranı, Grup II ve III'te (%100) kontrol grubundakilerden (%33.3±21.1) önemli düzeyde daha yüksek bulundu (P<0.01). Çiftleşme günleri (9.0±1.0, 4.7±0.7 ve 5.8±0.4 gün), gruplar (Grup I, II ve III, sırasıyla) arasında önemli düzeyde farklıydı (P≤0.01). Gebelik periyodu, Grup III'te (65.8±0.5 gün), Grup II (62.4±1.0 gün) ve kontrol dişilerindekinden (62.5±0.5 gün) daha uzundu (P<0.05). Bir batında doğan yavru sayısı, Grup II'de (4.6) Grup III (2.6) ve kontrol dişilerindekinden (3.0) daha yüksek olma eğilimindeydi (P=0.062). Son olarak, laktasyon periyodu, Grup III'te (105.2±2.6 gün) Grup II (94.6±1.7 gün) ve kontrol dişilerindekilerden (95.0±5.0 gün) önemli düzeyde daha uzundu (P<0.05). Elde edilen bulgulara göre; hem tek doz hem de bölünmüş doz eCG enjeksiyonunun üreme sezonu dışındaki endemik Van kedilerinde eşit seviyede ve yüksek oranda fertil östrusları uyarmada etkili olduğu kanısına varıldı.

Anahtar sözcükler: *eCG, Östrüs uyarımı, Fertilite, Van Kedisi, Anöstrüs sezonu*

İletişim (Correspondence)
+90 432 2251703/4583
atalayuslu@hotmail.com

INTRODUCTION

Turkish Van cats are endemic breed to Van province and attract public attention because of their white fur and typically colourful eyes (blue/yellow). They also behave friendly and typically like to play with water [1,2]. But, unfortunately this breed becomes increasingly extinct, mainly because of environmental factors, such as urbanisation.

Domestic queens are seasonally polyoestrous animals. They are long-day breeders such that a prolonged anoestrus occurs during short days, i.e. September-January in the northern hemisphere [3,4]. According to our earlier observations on this breed, the breeding season commences in January-February and continues until the autumn in eastern Turkey, with latitude of 38.03° N [4]. The length of oestrus cycle is around 27-30 d. Proestrus is not generally seen [5]. Oestrus length within the cycle continues for about 7-10 d. Oestrus may be determined by apparent receptivity of mounting tomcat and/or using the vaginal cytology [6,7]. The ovulation mechanism is a provoked type such that the LH peak (leading ovulation) occurs through the external stimuli [8,9]. Single mating can lead to sufficient LH secretion, but sometimes numerous matings are required. It is recommended that oestrous females should be mated 3 times a day, with 4 h interval [10]. Additionally, Terry et al.[11] reported that the greatest ovulation rates were obtained by mating 3 times daily, with 3 h interval commencing after the optimal time of oestrous behaviours (around the 5th d).

In queens, the ovarian stimulation can be manipulated using exogenous gonadotrophins. Since the eCG and hCG hormones have a longer half-life as compared to those of LH and FSH, the two former gonadotrophic hormones are preferred for induction protocols in wild cats. Indeed, the eCG and hCG reside within the blood stream for a minimum of 120 and 96 h, respectively [12]. Therefore, each hormone should reside within the body for a long time to achieve maturation/ovulation following single injection [13]. The ovarium of queens is quite sensitive against the eCG. Indeed, La Polt et al.[14] observed that its LH receptors were markedly increased through the eCG injection. However, it was also considered that season, age and even individual factors are effective co-factors of eCG [2,14,15].

Regarding the dose, Colby [16] reported that a dose of <100 IU eCG would not be effective for oestrus induction. However, an individual dose of 200 IU led to the occurrence of oestrus with 39.1% ovulation rate [17]. Nevertheless, a higher dose of 300-500 IU eCG resulted in superovulations, but this dramatically reduced the pregnancy rates [18]. In this respect, the reduction might be related to the excessive oestrogen production [19,20]. Additionally, the high number of follicular development as well as the luteinisation of premature follicles, follicular cyst formation, and immune response due to antibody formation may occur following its use. To overcome its side effects, queens should have a rest for at least 6 months before the next administration [21].

Given the background knowledge on the use of ecG in queens, it becomes logical that in endemic Van cats, gradualy becoming an extinct breed [2], healthy kittens may be obtained using the eCG only even during the non-breeding season. Considering the reports on single injection of 200 IU eCG leading rather low ovulation rate [17] or the minimum dose of 300 IU yielding dramatic reduction in pregnancy rate [18], single dose of 250 IU eCG chosen arbitrarily was considered to be sufficient enough to induce oestrus herein. Nevertheless, arbitrary split-doses of 75 IU, to be given for 5 d (with a higher total dose of 375 IU), were also presumed to be effective, such that it would also allow for sustaining eCG levels even longer (as compared to the single injection) within the circulating blood stream. This split injection protocol is further likely to lead a more effective (presumably longer) LH peak, with a greater ovulation rate eventually [8,9]. Split-low dose injections (instead of single-high dose)would also better avoid the likelihood of excessive stimulation of the ovarium [18-20] and antibody formation [21]. Therefore, the effects of single or split dosages (for 5 d) of eCG-based oestrus induction protocol were investigated upon the reproductive traits of Van cats during the non-breeding season.

MATERIAL and METHODS

Animals and Their Care

The study was conducted during the non-breeding season (mid-October onwards) using a total of 26 Van cats (18 queens and 8 tomcats), aged between 4 and 9 yrs-old. Cats were housed in the Van Cat Research Centre at Yuzuncu Yil University in Van, Turkey (altitude 42° 40′ and 44° 30′ East, longitude 37° 43′ and 39° 26′ North, altitude 1.727 m). For queens, the only criterion of selection was their pubertal maturity (*e.g.* oestrus, parturition). For tomcats, their selection was based on their previous reproductive performance, with sufficient libido and proven fertility. Vaccinations and antiparasitic drug administrations have being made periodically.

Animals were kept in six different management categories, in the rooms for; i) mature queens, ii) mature tomcats, iii) kittens, iv) mating couples, v) parturition, and vi) quarantine rooms. They were fed by a dry standard commercial diet (La Cat®, Israel) with water *ad libitum*.

eCG Administration

A total of 18 queens were divided into three experimental groups, involving 6 animals each, as follows:

Control, as Group I (n=6): 1 ml of 0.9% saline solution, as single *placebo* injection (i.m.),

Single eCG, as Group II (n=6): 250 IU of eCG injection (i.m.),

Split eCG, as Group III (n=6): a daily dose of 75 IU of eCG injection (i.m.) for 5 d.

In Groups II and III, there was one-week interval for the initiation of eCG injections. This was a precautious strategy, mainly because of small number of tomcats available (n=2-5 males, for each group), especially for treatment groups. Additionally, it was known that some females may have 'male preference' [22].

In Group I, single *placebo* injection (1 ml of 0.9% saline) was made in mid-October. Control animals were kept in the queen rooms with their own living environment shared by their female roommates.

In Group II, a single dose of 250 IU eCG (Folligon®, Intervet) injection was made on the 18th October.

In Group III, the first injection of 75 IU eCG was made on the 25th October and the injections were then continued for 5 days.

Animals were monitored three times a day (in the morning, mid-day/afternoon, and evening) for the behavioural oestrous signs. Females with presumed oestrus ('calling', tendency to approach cat keeper, leaning/rubbing neck to the person's legs and tail rolling/flagging) were introduced to tomcats and those showing receptivity were considered to be 'in oestrus'. Queens were hand-mated (by the same person's assistance) as long as they allow for mating.

Data Recording

Monitorisations were continued for 15 d after the hormonal administrations. Queens showing oestrous signs and those having mated already were transferred into the parturition rooms on the 50th d of their pregnancy.

Statistical Analysis

Data from reproductive parameters (oestrus/mating, pregnancy/parturition, litter size/survival, and lactation) were presented as mean ± SEM. The values from different induction groups (control, single or split eCG) were analysed by Pearson's correlation, regression analysis and one-way ANOVA using MINITAB [23]. Differences between the groups were considered significant when P<0.05.

RESULTS

The results of oestrus induction using single or split eCG administrations upon the reproductive traits of queens during the non-breeding season are given in *Table 1*.

The oestrus rates in Groups II and III were significantly higher (P<0.01) than those in controls. All the mating times (day of mating post-injection) were significantly (P≤0.01) different (earliest in single dose) between the groups. Pregnancy period was significantly (P<0.05) longer in Group III than both in Group II and controls. The litter (kitten) size tended (P=0.062) to be higher in Group II than those in Group III and controls. Finally, the lactation period was significantly (P<0.05) longer in Group III than both in Group II and controls. There was no further significant difference in any of the reproductive parameters studied.

DISCUSSION

Regarding the induction of oestrus in general, it is well known that although the GnRH could stimulate the oestrus in queens [24-26], but the results obtained may not always be satisfactory [18,27,28]. Herein, we achieved the pregnancy rates of 100% (in oestrous control queens, 2/6) or 83.3% (in both

Table 1. *The reproductive traits (mean±SEM) obtained following single or split eCG administration in Van cats during the non-breeding season*
Tablo 1. *Üreme sezonu dışında Van kedilerinde tekli veya bölünmüş eCG uygulamalarını takiben elde edilen üreme özellikleri (ortalama±SHO)*

Parameters	Experimental Groups			Statistics		
	Group I* (control) n=6	Group II (250 IU) n=6	Group III (75 IU d/for 5 d) n=6	F-ratio	P value	Significance
Previous parturition, %	83.3±16.7	83.3±16.7	66.7±21.1	0.28	0.761	NS
Oestrus, %	33.3±21.1 [a]	100±0.00 [b]	100±0.00 [b]	10.00	0.002	P<0.01
*Mating days, d	9.00±1.00 [c]	4.67±0.72 [a]	5.80±0.37 [b]	7.00	0.013	P≤0.01
*Mating number	6.50±2.50	7.83±1.54	7.40±0.87	0.14	0.874	NS
*Mating duration, d	2.00±0.00	1.83±0.31	1.40±0.25	0.91	0.433	NS
*Pregnancy period, d	62.50±0.50 [a]	62.40±1.03 [a]	65.80±0.49 [b]	5.63	0.026	P<0.05
*Parturition (fertility), %	100±0.00	83.3±16.7	83.3±16.7	0.16	0.856	NS
*Litter (kitten) size	3.00±0.00	4.60±0.68	2.60±0.40	3.85	0.062	NS (tendency)
*Survival of kittens, %	66.7±33.3	48.7±13.6	83.3±10.5	1.66	0.243	NS
*Lactation period, d	95.00±5.00 [a]	94.60±1.72 [a]	105.20±2.56 [b]	6.02	0.022	P<0.05

For Group I (control), only the queens showing oestrous signs (n=2) were considered for the analysis, [a-c] Means having different superscript within the same row significantly differ from each other (P<0.05),* ***NS:* *not significant*

eCG groups). Such a high pregnancy rates obtained in treated groups may be attributed to the longer half-life of eCG in blood and susceptibility of cat ovarium to the eCG, increasing the number of ovarian LH receptors [14] rather than a sudden rise in the P_4 [24,29,30] following the GnRH administration in breeding season. Nevertheless, Yu et al.[28], using a higher dose of eCG (400 IU) for stimulating the superovulations in breeding season obtained markedly lower rates of mature follicles and embryos, as compared to those receiving lower doses (50-100 IU). This was presumed to be a higher oestrogen levels occurred and abnormal tubal transport with higher eCG dose used.

The oestrus rates (100%) were markedly higher in treated queens than those in controls (33.3%). It was considered that, a lower oestrus rate obtained in controls would be related to pheromonal and behavioural interactions [26,31] with neighbouring eCG-treated females being kept together with controls.

Following the eCG administrations, there were marked differences of the time of oestrus response between the groups. The main reason for this might be due to longer half-life of eCG used [12], such that its half-life, as long as 120 h, should be long enough to stimulate the ovarium. In controls, the oestrous signs were observed as long as 9 d later following *placebo* injections. Herein, it was presumed that the intensity of individual interactions would be higher as the number of neighbouring oestrous females increased.

In our study, the average numbers of matings were 6.5, 7.8 and 7.4 in Groups I, II and III, respectively. Considering the previous reports of Terry et al.[11] and of Cain [10], these mating numbers given were successful enough to lead ovulations (sufficient fertility). In a further study, it was reported that only about 20% of females underwent ovulation following the mating [32]. Elsewhere, it was recorded that the most ideal ovulation was obtained by mating 3 times a day with 4 h interval [10]. The administration of 250-500 IU hCG at the time of mating decreased the number of matings, facilitating the prediction of pregnancy period [18]. A high rate of ovulation occurred when the dose was over 50 IU, but this increased the rate of degenerated ovum [33]. Tanaka et al.[34] reported that the injections of 100 IU hCG given 24 h apart on the 2nd and 4th d of oestrus could lead to 100% ovulation. Likewise, they achieved 91.7% ovulation rate after 25-27 h of 250 IU hCG injections. Additionally, Tsutsui et al.[35] observed 60% ovulation rate following 15-20 h of hCG injection. Moreover, in oestrous queens, a dose of 5-25 µg GnRH led to ovulation [36], and double dose of GnRH given on the 4th d, 12 h apart were also sufficient [37]. Finally, Wildt et al.[6] reported that the GnRH or its analogues could provide 20-100% ovulation rate. Nevertheless, one should also bear in mind that the GnRH injection is expected to lead the involvement of pituitary gland for endogenous LH relese, while the eCG injection provides the LH itself with no further need expected for the endogenous LH to be released. All these reports clearly show that the outcome of induction could easily be manipulated by varying doses of exogenous gonadotrophic hormone given.

For mating time (day post-injection), there were markedly different times (4.7, 5.8 and 9.0 d) of mating between single-, split eCG and control groups, respectively. A high (single) dose (250 IU) might have led to hastening the ovarian activity. However, in controls the oestrous signs appeared later, due presumably to a longer interaction required for sufficient pheromonal stimulation (possibly through odours of skin, urine/faeces, or other means) by roommate females [26]. Undoubtedly, all the animals within a given population have strong interrelationships. These interactions could be even more visible for reproductive activities especially in seasonally 'active' animals, such as in ewes [31,38]. Furthermore, in leopards, these female-to-female interactions are quite strong even leading to ovulation [26]. Therefore, we presumed that the queen-to-queen interactions might have led to 33.3% oestrus rate in control females that have being kept together with their 'hormone-treated' roommates.

Considering the pregnancy periods, markedly higher duration (65.8 d) was observed with split eCG, as compared to those with single dose and in controls (both around 62.5 d). It is known that, in multiple-kitten bearing pregnancies, the higher the kitten (litter) size achieved the shorter the pregnancy duration prevails [15].

Regarding the litter (kitten) size, no marked difference was observed between the groups. Nevertheless, the litter size (4.6) tended (P=0.062) to be higher in single dose group, than those in split eCG (2.6) and control groups (3.0). It is considered that both the injection of high dose and its long half-life presumably triggered a sudden development of numerous follicles at once. The response of ovarium in non-breeding season may be predicted, but this is not the case in mating season [26,39]. As mentioned earlier, feline ovarium is very sensitive to the eCG [14]. Hence, it was considered that in queens receiving a lower dose and daily/continuous injections, there might have been lower numbers of follicular development eventually yielding smaller litter size.

Finally, lactation period was markedly longer (105 d) in split eCG group than both in single dose and in control groups (both around 95 d). For this period, one has to consider the suckling kitten size (number). Indeed, this period was the longest in smaller size of kittens in split eCG group, while it was shorter in single dose group. Nevertheless, the numbers of surviving kittens in these groups were noticeable, such that the survival rates in single eCG, split dose and control groups were 66.7% (4/6), 48.7% (10/23) and 83.3% (11/13), respectively. It was considered that for parturitions resulting in multiple offsprings, the live weights of kittens born, their developmental stages and the amount of milk intake (suckling) could be limited. Hence, the survivability of large number of kittens may be low.

Overall, the present findings indicated that both single and multiple (split) eCG injections were equally very effective for induction of fertile oestrus in Van cats during the non-breeding season. Later occurrence of mating (post-injection), longer pregnancy period, smaller litter size and longer lactation period, as all achieved by the split eCG, resulted collectively in relatively superior survival rate of kittens. Undoubtedly, the early developmental advantages during the embryogenesis should also lead to longer survival. Therefore, in one hand, it may be concluded that the split injections (75 IU/daily) of eCG for 5 d appeared to be more effective than the single dose of 250 IU eCG treatment for obtaining live kittens, presumably through the longer periods of pregnancy and lactation. On the other hand, split doses for single eCG administration of 250 UI or single but increased dose (>250 UI) may also be preferred in future studies to see if a better outcome could be achieved in queens during the anoestrous season.

At last, to our knowledge, this study is the first report on the use of split eCG for induction of fertile oestrus in endemic Turkish Van cats during the non-breeding season.

REFERENCES

1. Güre A: Van Kedisi. **In,** Abdulkadiroğlu A, Yiğit M, Oğuzbaşaran B (Eds): Van Kütüğü. No: 8, Yüzüncü Yıl Üniversitesi, Van, pp. 722-726, 1993.

2. Odabaşıoğlu F, Ateş CT: Van Kedisi. Selçuk Üniversitesi Basımevi, Konya, 2000.

3. Hurni H: Daylength and breeding in the domestic cat. *Lab Anim,* 15, 229-233, 1981.

4. Gülyüz F, Alan M, Kaya M: Van kedilerinde vaginal smear yöntemiyle kızgınlık siklusu evrelerinin tanısı. *YYÜ Vet Fak Derg,* 5, 172-181, 1994.

5. Feldman EC, Nelson RW: Canine and Feline Endocrinology and Reproduction. Saunders, Philadelphia, USA, 1987.

6. Wildt DE, Chan SYW, Seager SWJ, Chakraborty PK: Ovarian activity, circulating hormones, and sexual behavior in the cat. I. Relationships during the coitus-induced luteal phase and the estrous period without mating. *Biol Reprod,* 25, 15-28, 1981.

7. Gay VL, Johnson LM: Patterns of LH release in the ovariectomized, estrogen treated cat following sequential copulations or GnRH injections. *Biol Reprod Suppl,* 26, 51 [Abstract], 1982.

8. Shille VM, Muriro C, Walker Farmer S, Papkoff H, Stabenfeldt GH: Ovarian and endocrine responses in the cat after coitus. *J Reprod Fertil,* 68, 29-39, 1983.

9. Concannon PW: Biology of gonadotrophin secretion in adult and prepubertal female dogs. *J Reprod Fertil Suppl,* 47, 3-27, 1993.

10. Cain JL: Drugs used to treat reproductive disorders. *Vet Clin North Am: Small Anim Pract,* 28, 395-410, 1998.

11. Terry LR, Barbara AW, Julie AL, Howard JG, Wildt DE: Effects of equine chorionic gonadotropin, human chorionic gonadotropin and laparoscopic AI on embryo, endocrine and luteal characteristics in the domestic cat. *Biol Reprod,* 57, 165-171, 1997.

12. Swanson WF, Wolfe BA, Brown JL, Martin-Jimenez T, Riviere JE, Roth TL, Wildt DE: Pharmacokinetics and ovarian-stimulatory effects of equine and human chorionic gonadotropins administered singly and in combination in the domestic cat. *Biol Reprod,* 57, 295-302, 1997.

13. Crichton EG, Bedows E, Miller-Lindholm AK, Baldwin DM, Armstrong DL, Graham LH, Ford JJ, Gjorret JO, Hyttel P, Pope CE, Vajta G, Naida M, Loskutoff NM: Efficacy of porcine gonadotropins for repeated stimulation of ovarian activity for oocytes retrieval and *in vitro* embryo production and cryopreservation in Siberian tigers *(Panthera tigris altaica)*. *Biol Reprod,* 68, 105-113, 2003.

14. La Polt PS, Oikawa M, Jia XC, Pargan C, Hsueh AJW: Gonadotropin-induced up- and down-regulation of rat ovarian LH receptor message levels during follicular growth, ovulation, and luteinization. *Endocrinology*, 126, 3277-3279, 1990.

15. Banks DR: Physiology and endocrinology of the feline estrous cycle. **In,** Morrow DA (Ed): Current Therapy in Theriogenology. pp. 795-800, WB Saunders Co., Philadelphia, USA, 1986.

16. Colby ED: Induced estrus and timed pregnancies in cats. *Lab Anim Care,* 20, 1075-1080, 1970.

17. Kanda M, Oikawa H, Nakao H, Tsutsui T: Early embryonic development *in vitro* and embryo transfer in the cat. *J Vet Med Sci,* 57, 641-646, 1995.

18. Cline EM, Jenning LL, Sojka NJ: Breeding laboratory cats during artificially induced estrus. *Lab Anim Sci,* 30, 1003-1005, 1980.

19. Cline EM, Randall PA, Oliphant G: Hormone mediated oviductal influence on mouse embryo development. *Fertil Steril,* 28, 766-771, 1973.

20. Stone SL, Richardson LL, Hamner CE, Oliphant G: Partial characterization of hormone-mediated inhibition of embryo development in rabbit oviduct fluid. *Biol Reprod,* 28, 766-771, 1977.

21. Swanson WF, Horohov DW, Godke RA: Production of exogenous gonadotrophin-neutralizing immunoglobulins in cats after repeated eCG-hCG treatment and relevance for assisted reproduction in felids. *J Reprod Fertil,* 105, 35-41, 1995.

22. Yom-Tov Y: The impact of domestic cat *(Felis catus)* on wildlife welfare and conservation: A literature review. http://www.tau.ac.illifescizoologymembersyom-tovinbalcats.pdf. 2003. *Accessed:* 18.12.2011.

23. Minitab: Version 11.2. MINITAB Inc., Pennsylvania, USA, 1996.

24. Graham LH, Swanson WF, Brown JL: Chorionic gonadotrophin administration in domestic cats causes an abnormal endocrine environment that disrupts oviductal embryo transport. *Theriogenology,* 54, 1117-1131, 2000.

25. Mattos MRF, Silva TFP, Pereira BS, Uchoa DC, Cardoso RCS, Silva AR, Domingues SFS, Ferreira MAL, Costa SHF, Costa-Filho JC, Silva LDM: Embryo transfer in the cat: First success in Latin America. Errata dos Anais do II Congresso Internacional de Medicina Felina - CIMFEL [on CD-ROM], junho 14-17 de 2001, Rio de Janeiro, pp. 2-3. Disponível em: www.ncvonline.com.br. 2010.

26. Kutzler MA: Estrus induction and synchronization in canids and felids. *Theriogenology,* 68, 354-374, 2007.

27. Pope CE: Embryo technology in conservation efforts for endangered felids. *Theriogenology,* 53, 163-174, 2000.

28. Yu XF, Cho SJ, Bang JI, Lee HS, Lee YS, Kwon TH, Deb GK, Kong IK: Effect of equine chorionic gonadotropin on the efficiency of super-ovulation induction for *in vivo* and *in vitro* embryo production in the cat. *Theriogenology,* 73, 413-420, 2010.

29. Roth TL, Wolfe BA, Long JA, Howard JG, Wildt DE: Effects of equine chorionic gonadotrophin, and laparoscopic artificial insemination on embryo, endocrine, and luteal characteristics in the domestic cat. *Biol Reprod,* 57, 65-71, 1997.

30. Barnes FL: The effects of the early uterine environment on the subsequent development of embryo and fetus. *Theriogenology,* 53, 644-658, 2000.

31. Zarco L, Rodriguez EF, Angulo MRB, Valencia J: Female to female stimulation of ovarian activity in the ewe. *Anim Reprod Sci,* 39, 251-258, 1995.

32. Wildt DE, Seager SWJ, Chakraborty PK: Effect of copulatory stimuli on incidence of ovulation and on serum luteinizing hormone in the cat. *Endocrinology,* 107, 1212-1217, 1980.

33. Goodrowe KL, Wall RJ, Wildt DE: Influence of gonadotropins on and developmental competence of *in vitro* fertilized feline follicular oocytes. *Theriogenology,* 29, 250 [Abstract], 1988.

34. Tanaka A, Takagi Y, Nakagawa K, Fujimoto Y, Hori T, Tsutsui T: Artificial intravaginal insemination using fresh semen in cats. *J Vet Med Sci,* 62, 1163-1167, 2000.

35. Tsutsui T, Sakai Y, Matsui Y, Sato M, Yamane I, Murao I: Induced ovulation in cats using porcine pituitary gland preparation during the non-breeding season. *Jpn J Vet Sci,* 52, 677-683, 1989.

36. Chakraborty PK, Wildt DE, Seager SWJ: Serum luteinizing hormone releasing hormone in the oestrous domestic cat. *Lab Anim Sci,* 29, 338-344, 1979.

37. Swanson WF, Bond JB, Steinetz BG, McRae MA: Fetal and neonatal development of domestic cats produced from *in vitro* fertilization and laparoscopic oviductal embryo transfer versus natural mating. *Theriogenology,* 55, 371 [Abstract], 2001.

38. Yıldız S, Uzun M, Kaya M, Uçar Ö, Çenesiz M: Effects of rams and luteal or follicular phase ewes on preovulatory LH surge characteristics in ewes. *Turk J Vet Anim Sci,* 28, 669-673, 2004.

39. Tsutsui T, Yamane I, Hattori I, Kurosawa N, Matsunaga H, Murao I, Kanda M, Hori T: Feline embryo transfer during the non-breeding season. *J Vet Med Sci,* 62, 1169-1175, 2000.