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Effect of deslorelin acetate treatment in oocyte recovery and in vitro embryo production in domestic cats

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Abstract

Objectives The present study investigated the effect of contraceptive treatment with deslorelin acetate on in vitro embryo production and oocyte recovery in domestic queens.

Methods Twenty-one mature domestic cats were used. Eleven queens (treated group) and one tom were kept in an experimental cattery, and 10 queens were privately owned (control group). When in interestrus or diestrus (day 0) a deslorelin acetate implant (Suprelorin, 4.7 mg/animal) was inserted into the subcutaneous tissue of the interscapular region in all queens in the treated group. After 6 months of treatment, all animals were ovariohysterectomized, and the ovaries were used for in vitro embryo production. Percentage of cleavage was determined 18 h after oocyte insemination and blastocyst formation was assessed on the eighth day of culture. The rate of cumulus-oocyte complexes (COCs) recovery was analyzed by an unpaired *t*-test. The cleavage and blastocyst rates were expressed as percentages and analyzed by Fisher's exact test. All analyses were performed using GraphPad Prism v5.0, with $P < 0.05$ set as the level of significance.

Results In the treated group, we recovered 8.3 ± 1.15 grade I COCs per queen; the cleavage rate was 60% and the blastocyst rate was 36%. In the control group, we recovered 18.4 ± 3.21 grade I COCs per queen; the cleavage rate was 55.97% and the blastocyst rate was 34%. Forty percent of treated females did not produce any blastocysts. In the treated group, we observed a significant decrease in COC recovery. Although there was no significant difference in cleavage and blastocyst rates between groups, 40% of treated females did not produce any blastocysts.

Conclusions Recovery of grade I COCs is negatively affected by deslorelin treatment in domestic cats. Regarding embryo production, new studies are still necessary to evaluate the success of this technique owing to the individual effect caused by deslorelin acetate.

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Introduction

The gonadotropin-releasing hormone agonist deslorelin promotes secure, efficient and long-term anestrus in queens. Although it is considered a reversible contraceptive, return to estrus after treatment is described as asynchronous.[1,2]

To overcome these asynchronies, estrus can be induced successfully in cats previously treated with deslorelin acetate after the removal of the implant and treatment with exogenous gonadotropins, indicating that ovary activity can be induced after deslorelin treatment.[3] Although all queens ovulated, and viable oocytes were recovered from all treated animals, there are no

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data regarding in vitro embryo production (IVP) using oocytes obtained from queens under contraceptive treatment.

Many authors have tried to demonstrate the seasonal influence on IVP in cats;[4–6] to the best of our knowledge no data are available about the influence of a long-term anestrus induced by contraceptives on IVP.

This is an important subject, especially owing to the large number of wild felids in captivity in the USA and Europe undergoing contraceptive treatment. IVP may be important in cases of sudden death or uterus disease in those females. Furthermore, reversibility after deslorelin treatment has been a challenge in species such as lions (AZA Reproductive Management Center, 2016, personal communication), and the possibility of embryo production in females currently under contraceptive treatment can be used as a tool to preserve genetic material from valuable individuals.

Based on that, the aim of the present study was to investigate the effect of contraceptive treatment with deslorelin acetate on IVP in domestic queens.

Materials and methods

All chemicals were obtained from Sigma-Aldrich, unless otherwise stated. All procedures were carried out in accordance with ethical standards of the committee on animal experimentation of São Paulo State University (UNESP).

Animals

Twenty-one mature mixed-breed domestic cats (2–3 years old) were used in this study. Eleven queens (treated group) and one tom were kept in an experimental cattery at the Department of Animal Reproduction and Veterinary Radiology, Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu, Brazil. The females were maintained in a 12 h light/day cycle with a 150 lux minimum. The animals were fed commercial cat food (Premier Gatos Adultos) and water was provided ad libitum. The other 10 queens were privately owned (control group); these females were maintained in their owners' houses and under natural light conditions (10–12 h of light/day). Only females who were proved breeders, that exhibited sexual activity and that never underwent to any contraceptive treatment were used as the control group.

Before the study began, all animals were vaccinated against common feline viruses (feline herpesvirus, feline calicivirus, feline panleukopenia) and rabies. Ultrasonography was used for the general examination of reproductive tracts.

Estrous cycle monitoring

Females from the treated group had their estrous cycle evaluated by vaginal cytology and behavior observations. The evaluations were conducted every 72 h by a single trained observer, at 9 am–10 am, as previously described.[3] Vaginal cells were classified according to the criteria proposed by Johnston et al.[7]

Contraceptive treatment

When the queens had a vaginal cytology characteristic of interestrus or diestrus (day 0), evidenced by <70% superficial epithelial cells, a deslorelin acetate implant (Suprelorin 4.7 mg/animal; Virbac) was inserted into the subcutaneous tissue of the interscapular region, after shaving and cleaning the area.[3] No general anesthesia was used for this procedure. After implant insertion, vaginal cytology was performed once each week in treated females. There was no intention in evaluate the seasonality of females in the control group; as such, no vaginal cytology was performed.

Oocyte recovery and in vitro maturation

After 6 months of treatment, all animals were submitted to general anesthesia for the ovariohysterectomy procedure. The ovaries were used for IVP. All surgeries were performed in June and July (winter in Brazil), including all females from the control group, to ensure they would be in anestrus – the same estrous phase as the treated females.

After removal, ovaries were kept in Dulbecco's phosphate-buffered saline (DPBS; Nutricell) at 4°C for a maximum of 2 h. Ovaries were sliced into a glass Petri dish containing 5 ml DPBS at 38°C using a scalpel blade to release cumulus-oocyte complexes (COCs). COCs were selected and classified, and only grade I COCs (uniform, dark cytoplasm surrounded by at least five layers of cumulus) were selected. Selected COCs were washed three times in Hepes-buffered minimum essential medium (Gibco) supplemented with bovine serum albumin (3 mg/ml), glutamine (2.0 mM), pyruvate (1.0 mM), cysteine (1.2 mM), streptomycin (100 mg/ml) and penicillin (100 UI/ml; Gibco).[6]

Selected COCs were incubated (10–30/400 µl) in a four-well dish (Nunc) containing 400 µl Dulbecco's modified essential medium (Gibco) supplemented with bovine serum albumin (BSA; 3 mg/ml), glutamine (2.0 mM), pyruvate (1.0 mM), cysteine (1.2 mM), streptomycin (100 mg/ml) and penicillin (100 UI/ml; all from Gibco), and bovine follicle-stimulating hormone (10 µg/ml; Folltropin V; Bioniche Animal Health), luteinizing hormone (1 µg/ml Lutropin V; Bioniche Animal Health), estradiol (1 µg/ml), insulin-like growth factor 1 (20 ng/ml) and basic fibroblast growth factor (10 ng/ml) for 32 h at 38°C in a humidified environment of 5% O_2, 5% CO_2 and 90% N_2.[6]

In vitro fertilization

After 32 h of in vitro maturation, oocytes were transferred to 90 µl drops of tyrode albumin lactate pyruvate (TALP)[8] supplemented with 20 µl/ml heparin. After

oocyte preparation, fresh semen (collected using an artificial vagina) was diluted in TALP solution supplemented with 6 mg/ml BSA, 1 mM pyruvate, 100 mg/ml streptomycin and 100 UI/ml penicillin and was centrifuged for 10 min (300 *g*).

The supernatant was discarded, and the remaining pellet was diluted in 30 µl TALP solution. Sperm motility was evaluated, sperm concentration was determined and then TALP was added to obtain a final concentration of 1×10^7 motile spermatozoa/ml. Subsequently, 10 µl (1×10^5 motile spermatozoa) of this suspension was used to inseminate each drop.

Embryo culture

Eighteen hours after insemination, the presumptive zygotes were denuded and the percentage of cleavage was determined. Every 5–10 zygotes were transferred to 100 µl drops of synthetic oviductal fluid (SOFaa) culture medium for culture over 3 days.[9] After that period, morulae were transferred to drops of the same culture medium supplemented with glucose (1.5 mM). At the eighth day of culture, blastocyst formation was assessed.

Statistical analysis

The rate of COC recovery was analyzed by an unpaired *t*-test. The cleavage and blastocyst rates were expressed as percentages and analyzed by Fisher's exact test. All analyses were performed using GraphPad Prism v5.0 (GraphPad), with $P < 0.05$ set as the level of significance.

Results

Contraceptive treatment

No pain or discomfort was observed in any of the treated queens during implant insertion. Three females showed estrous behavior, and had vaginal cytologies characteristic of estrus 1 week after receiving the implant. All signs of estrus disappeared within 1 week and were not observed for the next 6 months.

One female was removed from the experimental group owing to development of galactorrhea 2 months after contraceptive treatment began. This female was treated with 0.125 mg/kg metergoline (Sec Lac; Agener) every 12 h for 8 days. Despite the reduction of milk secretion, it did not cease, even after a second round of treatment. This female was not pregnant and had no signs of abortion. This queen was submitted to general anesthesia, and the implant was removed. Twenty days after implant removal, milk secretion ceased and the cat was ovariohysterectomized. No signs of pathology were observed in the uterus or ovaries. Furthermore, the ovaries from this female were not used for any steps of IVP.

Table 1 Grade I cumulus-oocyte complexes (COCs) (mean ± SEM) obtained per queen and rates of cleavage and blastocyst formation obtained in total from queens treated or not with deslorelin acetate (n = 10/group)

Group	Grade I COCs (mean ± SEM)	Cleavage	Blastocyst
Treated	8.3 ± 1.15[a]	50 (60%)	18 (36%)
Control	18.4 ± 3.21[b]	103 (56%)	33 (34%)

[a, b] $P < 0.05$

Oocyte recovery, in vitro fertilization and in vitro culture

All ovaries from both groups were morphologically normal, and no cysts, tumors or corpus luteum were observed. The total grade I COCs recovered and the cleavage and blastocyst rates are described in Table 1. The recovery rate was significantly higher ($P < 0.05$) for the control group, but no differences were observed between groups regarding cleavage and blastocyst rates. Despite this, it is important to note that 40% of the treated cats did not produce any embryos.

Individual results from the treated group are described in Table 2. In one cat (C), none of the grade 1 COCs cleaved, and in three other cats (B, D, G), none of the cleaved zygotes became blastocysts. All females from the control group produced at least two blastocysts.

Discussion

Recovery of grade I COCs from IVP was successfully performed from ovaries of cats under deslorelin acetate treatment; however, individual differences were observed among treated cats.

Initial ovarian stimulation, resulting in vaginal cytologies characteristic of estrus and estrous behavior, was expected and reported previously in domestic cats.[1–3] Ovarian quiescence was observed in all of our treated queens for 180 days, indicating that deslorelin acetate successfully suppressed ovarian activity and can be used as a contraceptive treatment in domestic cats for at least 6 months. This result was expected and consistent with previous reports.[1,2]

One queen had an episode of galactorrhea and was removed from the experiment. To the best of our knowledge, this is the first report of galactorrhea in a queen after deslorelin acetate treatment; this contraceptive has been used in domestic cats since 2001,[1] meaning that galactorrhea is a rare side effect, and is easily treated. Furthermore, no other side effects or unwanted effects were observed throughout the study.

Table 2 Grade I cumulus-oocyte complexes (COCs) and cleavage and blastocyst rates obtained from queens treated with deslorelin acetate (n = 10)

Queen	Grade I COCs (mean ± SD)	Cleavage	Blastocyst
A	12	8 (67%)	3 (38%)
B	5	2 (40%)	0 (0%)
C	3	0 (0%)	0 (0%)
D	10	5 (50%)	0 (0%)
E	11	6 (55%)	3 (50%)
F	10	6 (60%)	3 (50%)
G	2	1 (50%)	0 (0%)
H	8	6 (75%)	2 (33%)
I	11	8 (73%)	4 (50%)
J	11	8 (73%)	3 (38%)
Total	83	50 (60%)	18 (36%)

By the time of the ovariohysterectomy, all treated females were still under deslorelin treatment and reproductively inactive, as expected. All ovaries, from both groups, were morphologically normal (no cysts, tumor or morphological abnormalities were observed) and none of the females was in luteal phase.

In our study, the number of COCs recovered in the control group was similar to previous results described in the literature;[5,6,10–12] however, in the treated group, we observed a significant decrease in the recovery of COCs. Different factors may affect the number of COCs recovered, including the method used to retrieve the oocytes, season and ovarian status.

While in cattle a large number of aspiration methods have been tested to determine the best needle diameter and the vacuum pressure that should be used to retrieve the oocytes,[13] for domestic cats slicing is consider the methodology of choice to obtain COCs ex vivo.[5,6,10,11] Although individual variation may occur using slicing, the technique is apparently consistent enough to provide a similar recovery rate within the articles published.[5,10,11]

Apparently, ovarian status does not play an important role in COC retrieval in domestic cats.[5,6,10] Results regarding seasonal effect are still controversial; while some research has demonstrated an increased recovery rate during the breeding season,[6] others did not find any difference.[5,10] As the recovery technique and season were the same for both groups in our study, we believe that the contraceptive treatment is responsible for the low COCs obtained from treated queens.

Although the number of COCs was lower in treated group, the cleavage and blastocyst rate did not differ among groups. Cleavage and blastocyst rate were consistent in females in the control group, and only one female had lower rates than described in literature. However, in cats in the treated group we observed a marked individual variance, and four females were not able to produce any blastocysts.

Data regarding the factors that may influence the meiotic competence and in vitro development of domestic cat oocytes are still controversial. In domestic cats, about 50–60% of oocytes achieve nuclear maturation after in vitro culture and <60% of matured oocytes are fertilized.[5,6,10] It seems clear that culture conditions,[14] morphological quality of oocytes[15–17] and storage conditions[5,6] play important roles; however, there is no consensus on the importance of reproductive status, ovarian conditions and breeding season.[5,6,10] As most research is performed in different laboratorial conditions, latitude and season, it is difficult to compare results.

Both the in vitro maturation and IFM protocols used in our study were correct for the species and the results obtained from cats in the control group are similar to those already described for domestic cats.[5,6] We believe that under the conditions of our study, contraceptive treatment with deslorelin acetate seemed to be responsible for both the positive and negative results obtained individually in cats in the treated group. While it was possible to obtain a high rate of cleavage and blastocyst production in some females, in other females we were not able to produce embryos.

As mentioned previously, there is no consensus regarding reproductive status, ovarian conditions and breeding season. All females from the treated group were maintained under the same conditions and were spayed during the same season. Apparently, the results obtained in cats B, C, D and G were due alterations of developmental competence of the oocytes, but it is unclear which mechanisms the contraceptive treatment affected those oocytes.

It is important to note that an excellent blastocyst rate was obtained from the rest of the treated group, similar to the rate in the literature and individually even better than the numbers described by our laboratory.[8] It is well known that short-term contraception is advantageous in women undergoing superovulation protocols in an in vitro fertilization program,[18,19] and in domestic cats, short-term contraception can also improve superovulation.[3] This improvement due to short-term contraception is attributed to the fact that a quiescent ovary stimulated by exogenous gonadotropins probably responds with a predictable number of ovulations,[20] and, possibly, also plays a unknown role improving IVP.

It is important to note that one of the most remarkable characteristics of deslorelin acetate is the individual and interspecies variation,[1–3,20–22] which explains the differing results within the treated group.

Conclusions

Deslorelin treatment negatively affects the recovery rate of grade I COCs, but not the quality of COCs as the

cleavage and blastocyst rates were high and similar to control cats. Regarding embryo production, new studies are still necessary to evaluate the success of this technique, owing to the individual effects caused by deslorelin acetate. However, the females that were able to produce embryos had a good rate of production. We strongly encourage the use of ovaries from treated females, in cases of sudden death, for IVP as an attempt to produce a final litter from those females.

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