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Review Article

Current Diagnostic and Therapeutic Approaches to Ovarian Remnant Syndrome in Queens

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ÖZET

Ovarian Remnant Syndrome (ORS) is a disease characterized by clinical findings arising from remnant ovarian tissue following a spaying surgery. Affected queens may exhibit behavioral signs of estrus, and in chronic cases, severe complications such as stump pyometra, mammary tumors, or granulosa cell tumors may develop, significantly impairing the animal's quality of life. Therefore, surgical removal of ovarian remnants is highly recommended. Due to its low prevalence, ORS remains a subject of ongoing research aimed at clearly identifying its causes, accurate diagnosis, and the most effective surgical treatment methods. Thus, this review aims to provide a comprehensive overview of the literature regarding the etiology, clinical presentation, diagnostic methods, including AMH measurement as a new and trending technique, and treatment options for ORS in queens. Ultimately, a deeper understanding of ORS in queens by clinical veterinarians may contribute to better outcomes and quality of life for affected queens.

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Dişi Kedilerde Ovaryan Remnant Sendromuna Yönelik Güncel Tanı ve Tedavi Yaklaşımları

ABSTRACT

Ovaryan Remnant Sendrom (ORS), kısırlaştırma ameliyatı sonrasında kalan ovaryum dokusuna bağlı olarak gelişen klinik bulgularla karakterize bir hastalıktır. Etkilenen dişi kedilerde kızgınlık davranışları gözlemlenebilir ve kronik vakalarda, ciddi komplikasyonlar olarak stump pyometra, meme tümörleri veya granüloza hücre tümörleri gelişebilir; bu durum hayvanın yaşam kalitesini önemli ölçüde düşürebilir. Bu nedenle, kalıntı ovaryum/dokusunun operatif olarak uzaklaştırılması önemle tavsiye edilmektedir. Görülme sıklığının düşük olması nedeniyle, ORS'nin nedenlerinin, doğru teşhis yöntemlerinin ve en etkili cerrahi tedavi yöntemlerinin net olarak belirlenebilmesi amacıyla araştırmalar halen devam etmektedir. Bu derleme, ORS'nin kedilerdeki etiyolojisi, klinik bulguları, AMH ölçümü gibi yeni ve gelişmekte olan bir teknik de dahil olmak üzere tanı yöntemleri ve tedavi seçenekleri ile ilgili literatürün kapsamlı bir özetini sunmayı amaçlamaktadır. Bu sebeple, ORS'nin Veteriner Hekimler tarafından daha iyi anlaşılması, etkilenen dişi kediler için daha iyi sonuçlara ve yaşam kalitesinin artırılmasına katkı sağlayabileceği düşünülmektedir.

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INTRODUCTION

Spaying is recommended to prevent, uncontrolled population growth in both stray queens and bitches, unwanted pregnancies, potential reproductive system-related diseases and the behavioral problems associated with estrus. Additionally, it minimizes the risk of infection transmission that can occur through social encounters facilitated by estrus behavior. Thereby, it improves quality of life and reduces the risk of illness. Two operative methods, ovariohysterectomy and ovariectomy, are most preferred methods for spaying (Sontas et al., 2007). Ovarian Remnant Syndrome (ORS) is characterized by clinical estrus findings arising from remnant ovarian tissue following a spaying surgery (Oliveira et al., 2012). In veterinary medicine, early reports of ORS in bitches and queens appeared in the 1990s, notably by Wallace (1991), who described the syndrome in both bitches and queens. ORS can occur as a result of incomplete removal of ovaries during ovariohysterectomy or ovariectomy surgery, and it can also be observed with dropping of ovarian tissue inadvertently into the abdomen during surgery (Feldman & Nelson, 2004; Romagnoli, 2004; Wallace, 1991) leading to ovarian autotransplantation (Ball et al., 2010; Fontes & McCarthy, 2020). While often debated, there is limited evidence to suggest that congenital ectopic ovarian tissues could potentially contribute to the development of ORS in domestic animal species (McEntee, 2012). In cases where estrous behavior is observed after spaying, it is often attributed to estrogen secretion from the adrenal glands, which are recognized as the second most significant source of sex steroid hormones in the body after the ovaries and testes. However, only one case report in the literature has documented adrenal-derived estrous behavior in a queen, and it was associated with an adrenocortical carcinoma. (Meler et al., 2011).

Clinical signs in animals affected by this syndrome may take months or years to appear (Flock et al., 2022). It is important to note that these clinical findings can vary significantly from one case to another. The most common clinical findings consist of the behavioral signs of estrus or proestrus, include vocalizations, lordosis, attractivity to males, and even permit copulation (Feldman & Nelson, 2004; Wallace, 1991). However, these estrus behaviors may not always be clearly observed in every suspected case of ORS. In addition, this syndrome can lead to potential future pathologies such as pyometra (stump), ovarian tumors, and hyperandrogenism due to the ongoing production of ovarian hormones by the remnant ovarian tissue (Ball et al., 2010; Demirel & Acar, 2012; Jones et al., 2019) as in intact animals.

Accurate diagnosis is crucial, as the success of treatment is closely linked to the effectiveness of the diagnostic process. To achieve a definitive diagnosis, a multifaceted diagnostic approach is often required, including thorough evaluation of the patient's history, assessment of clinical signs, estrus staging through vaginal cytology, hormone stimulation protocols, abdominal ultrasonography, measurement of luteinizing hormone (LH) levels, and, when necessary, exploratory laparotomy (DeNardo et al., 2001; Feldman & Nelson, 2004; Krecic et al., 2018). This diagnostic process which needs variety of examinations and interpretation of the results can be influenced by factors such as the clinician's experience, intensity of clinical findings and characteristics of the reproductive cycle. Recently, Anti-Müllerian Hormone (AMH) has emerged as a novel and promising biomarker for the diagnosis of ORS. However, the diagnostic utility of AMH in queens remains controversial in the literature, which contributes to the ongoing challenge of accurately diagnosing ORS (Johnson et al., 2023).

Surgical treatment, which involves laparotomic or laparoscopic methods, plays a crucial role in the management of ORS, serving as both a diagnostic tool and the definitive treatment option. This method allows for the precise localization and removal of remnant ovarian tissue during the procedure (Oliveira et al., 2012).

After the clinical signs appear, some veterinarians prefer to suppress the clinical symptoms instead of to treating ORS by performing surgical removal of the tissue. Progesterone is the most commonly used hormone for this purpose, however do not resolve the problem and can also lead to side effects such as profound adrenocortical suppression, adrenal atrophy, transient diabetes mellitus, polydipsia/polyuria, behavioral changes, increased weight, endometritis, cystic endometrial hyperplasia, pyometra, mammary hypertrophy, neoplasia and hepatotoxicity (Plumb, 2011).

Clinically, estrus manifestations are typically more pronounced and perceived as more distressing by owners, the majority of ORS cases presented to veterinary clinics involve queens. This clinical trend, coupled with the challenges in diagnosing and treating ORS, emphasizes the necessity for this article to provide a comprehensive approach to ORS and a review of current and up-to-date diagnostic and treatment methods in queens.

OVARIAN REMNANT SENDROME IN QUEENS

Etiology of ORS

ORS is more prevalent in queens than in bitches and can occur either bilaterally or unilaterally, with a similar frequency of occurrence for both (Miller, 1995). The most prevalent cause of estrus-like behavioral and physical signs following spaying is the presence of remnant ovarian tissue that has resumed folliculogenesis and estrogen production (Feldman & Nelson, 2004). This condition almost always results from surgical technique errors, where one or both ovaries and/or their remnants remain or were inadvertently dropped into the peritoneal cavity. The retained ovarian tissue can remain/become hormonally active and may even produce follicles that can ovulate.

This condition may be associated with the surgeon's preferences on a small incision, which can limit visibility, access, or proper ligation of the ovaries, or it may result from the surgeon's lack of experience leading to incorrect ligation. Also, the anatomical positioning of the right ovary (Miller, 1995; Johnston et al., 2001, Sontas et al., 2007; Wallace, 1991) and it's shorter suspensor ligament makes difficult to take the ovary excision, creating a predisposing situation for ORS (Sontas et al., 2007). Therefore, some surgeons may choose to perform the procedure from the right flank due to the more cranial location of the right ovary and the lack of omental coverage over the viscera (McGrath et al., 2004), while the left flank is more commonly preferred as it allows the dominant hand to apply tension to the ovarian pedicle more effectively even it is reported (Demirel & Acar, 2012) as left lateral flank laparotomy can make accessing the right ovary more challenging, potentially leading to incomplete removal.On the other hand, it is reported that ORS occurred following laparoscopic ovariohysterectomy in a queen (Properzi et al., 2018).

Another cause of ORS is the dropping of ovarian tissue inadvertently into the abdomen during surgery (Feldman & Nelson, 2004; Fontes & McCarthy, 2020; Romagnoli, 2004; Wallace, 1991). If a piece of ovarian tissue accidentally falls into the abdomen during surgery, it can become revascularized by the omentum or serosa of visceral structures in the abdomen, thereby gaining normal ovarian function (DeNardo et al., 2001; Feldman & Nelson, 2004; Romagnoli, 2004; Sontas et al., 2007).

The occurrence of estrus after ovariohysterectomy/ovariectomy may be attributed in some cases to the presence of an accessory ovary or to ovarian tissue extending into the ligamentum proprium of the ovary (McEntee, 2012). Accessory ovaries, which can be small and located in the proper ovarian ligament, are separated from the main ovary by connective tissue. Although the main ovary is removed the accessory ovary is functional. Ovarian remnants can continue cycling even in the absence of the uterus. It is important to note that accessory ovarian tissue can become active after the removal of ovaries (McEntee, 2012; Sontas et al., 2007).

Clinical Signs of ORS

The duration from spaying to the appearance of clinical signs in queens can vary significantly, reported as ranging from 17 days to 9 years (Miller, 1995). The initial onset of ORS also varies depending on the time required for revascularization of the remnant tissue (DeNardo et al., 2001) and of course, on the seasonal character of their reproductive activities in queens (Wallace, 1991).

These signs of estrus in ORS cases commonly include vocalization, rolling on the ground, head rubbing, lordosis, tail deviation, nymphomania, and even permit to copulation (Feldman & Nelson, 2004). In some queens, these estrus symptoms can be very pronounced, however, they may not always be clearly observed in every suspected case of ORS. Other reported findings associated with ORS include mammary gland development, pollakiuria, stranguria, recurrent urinary tract infections, poor coat quality or alopecia, polyuria/polydipsia, polyphagia, chronic vaginitis, and false pregnancy (Ball et al., 2010). Although rare, some conditions may result in hyperestrogenism, which is linked to generalized alopecia and hyperpigmentation (Ball et al., 2010). Furthermore, remnant ovarian tissue may become cystic or develop ovarian tumors, potentially leading to a range of clinical manifestations (Ball et al., 2010). This syndrome also has the potential to cause secondary complications in the future, such as stump pyometra, and hyperandrogenism, all of which are associated with the persistence of functional ovarian tissue (Ball et al., 2010; Demirel & Acar, 2012; Jones et al., 2019).

Diagnosis of ORS

Despite the clinical signs associated with estrus behavior, the definitive diagnosis of ORS is challenging because the onset of this behavior may not always be clear depend on the amount of remnant functional tissue within the abdomen (Feldman & Nelson, 2004) and certain tumors reported in queens which have secretion of sex hormones such as adrenocortical carcinoma (Meler et al., 2011) and cystadenomas (Fontes & McCarthy, 2020). Anamnesis, clinical findings, vaginal cytology, hormone stimulation test, ultrasound findings, measuring luteinizing hormone (LH) levels and exploratory laparotomy are currently used for the diagnosis of ORS (DeNardo et al., 2001; Feldman & Nelson, 2004; Krecic et al., 2018). However, diagnostic methods may have advantages and limitations over one another (Table 1).

Table 1. *Comparison of the advantages and limitations of methods used for the diagnosis of Ovarian Remnant Syndrome in queens.*

Method	Advantages	Limitations	References
Vaginal Cytology	• Minimally invasive • Useful for detecting estrogen influence	• Only be applied during the estrus phase • Not specific to ORS • Can give false positives in adrenal-origin estrogen cases	Flock et al., 2022; Turna Yilmaz et al., 2015
Estradiol/Progesterone Assay	• Can identify hormonal activity • Progesterone useful when AMH is inconclusive	• Hormonal fluctuations can affect accuracy • Requires timed sampling for best interpretation	DeNardo et al., 2001; Place et al., 2019; Turna Yilmaz et al., 2015; Wallace, 1991
Ultrasound Imaging	• Non-invasive • May detect large or cystic remnants	• Operator-dependent • May miss small or inactive remnants	Flock et al., 2022; Mullikin et al., 2022
Estrus Stimulation Test	• Functional confirmation of tissue • Useful in ambiguous cases	• Time-consuming • Requires repeated sampling • May be inconclusive in luteal phase remnants	Axner et al., 2008; Gozer et al., 2023; Turna Yilmaz et al., 2015
Serum AMH Assay	• Minimally invasive • Relatively stable across estrous cycle • Useful screening tool	• May be low if remnant tissue is mostly luteal • Limited sensitivity in some cases	Axnér & Holst, 2015; Flock et al., 2022; Gozer et al., 2023; Place et al., 2019; Turna Yilmaz et al., 2015; Walter, 2020
Exploratory Laparotomy	• Definitive diagnosis and treatment	• Invasive • Requires general anesthesia • Final option approach	Flock et al., 2022; Walter, 2020

A thorough anamnesis should be conducted. The recurrence of estrus signs, despite the expectation that such symptoms would resolve following ovariohysterectomy, is a commonly reported finding in the anamnesis of nearly all cases. The persistence of estrus behaviors previously observed by the owner before spaying, appearing with similar frequency and intensity postoperatively, should be regarded as a strong indicator of ORS. However, estrus-related signs may not always be recognized or associated with ORS by the owner, particularly when they are mild or intermittent. Therefore, the observations not related with estrus must be also thoroughly investigated during anamnesis such as vulvar discharge, palpable mammary masses or asymmetry, and marking behavior.

Vaginal cytology is a method that can be useful only when the animals in estrus, and it's basic principle based on vaginal cornified epithelial cells can vary with the estrous cycle (Wallace, 1991). During proestrus, the number of basal and parabasal cells decreases, while the number of large intermediate and superficial cells increases. Parabasal and basal cells have round, large nuclei and a normal appearance; large intermediate cells are smooth, round, and have smaller nuclei compared to parabasal cells; superficial cells are large epithelial cells with irregular shapes and pyknotic nuclei, which may be difficult to distinguish from the cytoplasm or appear pale. These cells indicate estrogen activity throughout the estrous cycle. Vaginal cytology should ideally be done during estrus, as the presence of 80-90 % superficial cells at this stage indicates ovarian activity (Johnston et al., 2001). For the cases that not in estrus, it can be done following estrus stimulation test.

Estrus stimulation test allows the detection of ovarian tissue by measuring estradiol before and after application with Gonadotropin Releasing Hormone (GnRH) -analog stimulation test if no signs of estrus are found during examination. The researchers designed the study based on the fact that GnRH induces the release of FSH, which stimulates estradiol production by granulosa cells in ovarian follicles (Axnér et al., 2008). To investigate this, after an initial blood sample was taken, , Buserelin 0.4 µg/kg was administered intramuscularly to 11 spayed queens and 11 intact queens, and estradiol measurement was performed 2 hours later, by taking second blood sample. After the stimulation test, the average estradiol level increased in the intact group, while no increase was observed in the spayed group. After GnRH administration, the highest estradiol level measured was 9 pmol/L in the spayed group and 12 pmol/L in the intact group (Axnér et al., 2008). Therefore, if remnant ovarian tissue is left behind, the estrogen levels will be increase in the estrus stimulation test results.

In queens even ovulation can also occur without any external provocation, however, they are known as an induced ovulator species, and if the queen does not mate, in most of the cases the progesterone level remains low (Shille & Olson, 1989). Therefore, measuring progesterone during a period when estrus signs are not observed is not reliable for determining the presence of ovarian tissue. During estrus, ovulation induction can be achieved with administrations of a single dose GnRH (25 µg/queen) or human Chorionic Gonadotropin (hCG, 250 IU/queen) (Kutzler, 2007). A rise in serum progesterone levels measured 1-3 weeks after the administration indicates the presence of ovarian tissue (DeNardo et al., 2001, Wallace, 1991). If a queen has mated or undergone ovulation induction, serum progesterone levels remain above 2 ng/mL for at least 30 days following ovulation (Shille & Olson, 1989) and it's a critical indicator in the diagnosis of ORS, as they demonstrate active secretion from luteinized follicles. In a specific case report, a spayed domestic shorthair queen was evaluated for recurring estrus behavior following ovariohysterectomy. Serum testing indicated a progesterone concentration of 6.76 ng/mL, which aligned with the presence of an ovarian remnant sendrome (Fontes & McCarthy, 2020). In addition, it should be noted that progesterone levels increase after adrenocorticotropic hormone (ACTH) treatment and in stressful situations (Chatdarong et al., 2006).

Abdominal ultrasonography is one of the examinations utilized for diagnosing ORS (Mullikin et al., 2022). During ultrasonographic examination, ovarian remnants may appear as round, hyperechoic or cystic structures, occasionally accompanied by septations or fluid, and are often located caudal to the kidneys (Ball et al., 2010). Assessment of the uterine stump should also be performed during the examination; any increase in the stump may indicate the presence of functional ovarian remnants (Weedon & Kustritz, 2020). The diagnosis of ORS can be more clearly and easily established, especially in abdominal ultrasonography, with the presence of follicles or corpora lutea (Ball et al., 2007). In one study, ultrasonographic examination was performed and the presence of remnant ovarian tissue was confirmed on 11 out of 13 queens (Flock et al., 2022). However, the effectiveness of ultrasound in diagnosing depends on several factors: the expertise of the operator, the stage of animal's estrous cycle phase and the size of the ovarian remnant. These variables can significantly impact the accuracy and reliability of ultrasound as a diagnostic tool.

Recent studies have increasingly proposed AMH measurement is the new approach and trending diagnostic method for ORS (Flock et al., 2022; Fontes & McCarthy, 2020). This hormone, which has a glycoprotein structure, produces by the granulosa cells of preantral and small antral follicles from birth to menopause and reflects the ovarian antral follicle reserve in women (Visser et al., 2006). It has been reported that the average AMH level in queens with ORS is significantly higher compared to spayed queens and lower than in intact queens (Flock et al., 2022). Although AMH has been regarded as a reliable and minimally invasive biomarker for detecting ovarian remnants (Flock et al., 2022; Place et al., 2019, Turna Yilmaz et al., 2015), more recent evidence suggests that its diagnostic sensitivity may vary depending on the structure and size of the remnant tissue (Gozer et al., 2023). Gozer et al. (2023) observed that mean serum AMH levels in queens with ORS were higher than in spayed queens but not significantly different from those with ovarian cysts. It also has

been described that AMH may be low when the remaining ovarian tissue contains mostly corpora lutea in a study including bitches (Place et al., 2019). This suggests that the AMH concentration may also be low in queens when the remnant ovarian tissue mainly consists of luteal tissue, as described in bitches. Therefore, an additional progesterone measurement may be helpful in the diagnostic approach to ORS. In a previous study conducted in 2015, the ELISA method was used to assess spaying status in queens via measuring serum AMH levels and the lowest AMH value for spayed queens was measured at 0.14 ng/mL (Axnér & Holst, 2015). This value is indicated as the lowest level that the test can measure. In another study (Flock et al., 2022), all spayed queens were found to have an AMH concentration below the lower limit (≤0.01 ng/ml) using murine anti-AMH antibodies in a chemiluminescent immunoassay. The AMH level greater than 0.02 ng/ml in queens with ORS indicates the presence of functional ovarian tissue (Flock et al., 2022). Walter (2020) provided further support for the clinical applicability of AMH testing by noting its relative stability across different stages of the estrous cycle in queens. This characteristic enhances its practicality for use in unscheduled or single-time-point sampling scenarios for ORS.

Treatment of ORS

Surgical removal of the remnant ovary/ovarian tissue is the one and only treatment option. It's essentially involving laparotomic or laparoscopic methods for the both localization and removal of remnant tissue. Identifying the correct timing for surgical intervention is crucial for a successful procedure. Ideally, the surgical approach should be performed during diestrus or estrus, as the presence of follicles or corpus luteum and collateral vascularization ensures better visualization and manipulation of ovarian tissue (Oliveira et al., 2012). Clinical signs will completely disappear within a few days or weeks after the removal of remnant ovarian tissue (Wallace, 1991).

While diagnostic laparotomy is regarded as a final option for diagnosis, it is also essential for achieving definitive treatment. To facilitate a thorough procedure, the midline incision should be made more cranially compared to routine incision in spaying procedure. During the surgery, the caudal aspect of both kidneys, mesometrium, omentum and abdominal wall should be thoroughly examined for the localization of the existing ovary/remnant tissue. Additionally, should be inspected for any pathological condition related to the uterus, as the uterus and uterine horns may also have been retained (Sontas et al., 2007). Miller (1995) reported that remnant tissue in ORS is most commonly found in the pedicle of the ovary. If remnant ovary cannot be identified during the surgery, granulation tissues in the pedicle of the ovary should be examined (Sontas et al., 2007). In addition, the careful examination of ligated ovarian pedicle shouldn't be overlooked for the possibility of the presence of accessory ovaries, especially in queens (Miller, 1995).

The development of minimal invasive laparoscopic surgery (MILS) may advance the surgical treatment of ORS. Park et al. (2015) conducted that laparoscopic ovariectomy was considered an appropriate method for the surgical management of ORS in queens. MILS provides patients with less post-operative pain and better recovery (Devitt et al., 2005, Van Nimwegen et al., 2018). Two successful laparoscopic interventions for ORS have been reported in a queen (Brun et al., 2006) and bitches (Beck et al., 2004, Van Nimwegen et al., 2018). Video laparoscopy can facilitate the detection of remnant tissue. Advantages of this surgical procedure over traditional laparotomy include magnification of the view (i.e., better visualization of intraperitoneal structures), minimal surgical trauma, shorter postoperative hospitalization, and less pain. As the procedure may be complicated by adhesion formation, the experience of the surgeon significantly impacts the success rate of laparoscopic ORS treatment (Brückner, 2016).

In cases where laparotomy is not feasible, estrus suppression through medical approach is often considered by the clinicians; however, it must be emphasized that while drugs may suppress the symptoms of ORS, they do not resolve the problem and can lead to side effects. These possibilities should be considered when owners reject exploratory laparotomy and the risks of surgery, when the localization of the ovary cannot be determined, or financial constraints that may prevent the owner from proceeding with operative management. Generally, medical intervention relies on the use of exogenous progestagens to suppress behavioral signs of estrus. However, it is important to note that these medical interventions primarily address only the behavioral signs associated with estrus and do not eliminate the underlying issues of ovarian remnants. Additionally, new treatments being investigated to suppress estrus, such as GnRH agonists, GnRH antagonists, immunization against GnRH, LH, or LH receptor, and GnRH-toxin conjugates, may offer temporary symptom relief but fall short of providing a definitive cure for ORS (Oliveira et al., 2012). However, it is important to note that these

medical interventions primarily address only the behavioral signs associated with estrus and do not eliminate the underlying issues of ovarian remnants and can lead to side effects.

CONCLUSION

Although ovariohysterectomy/ovariectomy is a commonly performed surgical procedure for spaying, it may result in the inadvertent retention of hormonally active ovarian tissue, leading to ORS, a condition that presents significant diagnostic and therapeutic challenges. When the recent approaches to ORS be considered; measurement of AMH stands out as a practical and non-invasive diagnostic tool, as it requires only a single blood sample and offers rapid results independent of the estrous cycle—making it highly advantageous for clinical decision-making. This article also contrasts operative and non-operative treatments of ORS and operative removal of the remnant tissue remains the definitive treatment. Advances in laparoscopic techniques offer a less invasive alternative to traditional laparotomy, with benefits such as improved visualization, faster recovery, and reduced postoperative pain. However, the success of laparoscopy significantly depends on the surgeon's experience and the presence of adhesions, which may complicate the procedure. In conclusion, a comprehensive understanding of ORS in queens by clinical veterinarians may contribute to more successful outcomes and an improved quality of life for affected queens.

CONFLICT OF INTEREST

The authors do not declare any conflicts of interest.

AUTHOR CONTRIBUTION

All authors have contributed equally.

ETHICAL APPROVAL

The manuscript does not require ethics committee approval and the Authors stated that it was conducted in accordance with the relevant scientific, ethical and citation rules.

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