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Effects of GnRH Antagonists vs Agonists in Domestic Carnivores, a Review

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Contents
Gonadotrophin-releasing hormone (GnRH) stimulates the pituitary secretion of both luteinizing and follicle-stimulating hormones, and thus controls the hormonal and reproductive functions of the gonads. GnRH analogs, which include agonists and antagonists, have been produced by amino acid substitutions within the native GnRH molecule resulting in greater potency and a longer duration of effectiveness. While the initial antagonists produced significant side effects, more recent potent, long-acting, water-soluble, low histamine-release third-generation compounds such as cetrorelix, abarelix, azaline B and acyline have appeared. Differently to GnRH agonists, antagonists competitively block and inhibit GnRH-induced GnRH receptor gene expression leading to an immediate, dose-dependent, pituitary suppression without an initial stimulation of the gonadal axis. The aims of this review are to compare the effects of GnRH agonists vs antagonists and to describe the existing literature concerning new antagonists in domestic carnivores. In male dogs, a single subcutaneous dose of acyline safely and reversibly decreased serum gonadotrophins and testosterone concentrations for 9 days and prevented physiological response of gonadal the axis to agonistic challenge for 14 days. The same protocol reversibly impaired spermiogenesis, spermatocytogenesis and semen quality in both cats and dogs. In females, third-generation GnRH antagonists prevented ovulation and interrupted pregnancy in canids but not in felids. During anestrus, a single acyline injection exhibited limited prevention of the 'flare-up' effect in GnRH agonist-implanted bitches. Although GnRH antagonists appear to have a promising future in domestic carnivores reproduction, the information is still scarce and further work is needed before they can be widely recommended.

Introduction

Pulsatile gonadotrophin-releasing hormone (GnRH) stimulates the pituitary secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and thus controls the hormonal and reproductive function of the gonads (Hull and Kenigsberg 1987). GnRH analogs, which include agonists and antagonists, have been produced by amino acid substitutions within the native GnRH molecule resulting in greater potency and a longer duration of effectiveness (Vickery 1985; Gobello 2007).

Continuous administration or long-acting GnRH agonists override the physiologic pulsatile GnRH secretion leading to desensitization of pituitary GnRH receptors and the corresponding inhibition of gonadotrophin and gonadal hormones secretion (Vickery 1985; Vickery et al. 1989). The major drawback of agonists is that they initially stimulate the gonadal axis, which results in a surge in pituitary and gonadal hormones for 2–3 weeks before chemical castration is achieved. This initial stimulation may produce a flare in clinical symptoms in patients with hormone-dependent disease. Long-acting (1–12-month) formulations of GnRH agonists (e.g. leuprolide, buserelin and deslorelin) are available. Differently to agonists, GnRH antagonists bind to on the same receptors as native GnRH in the pituitary but are devoid of stimulatory activity. Receptor occupancy by the antagonists blocks the receptor causing an immediate, dose-dependent, suppression of gonadotrophin release (Heber et al. 1982). The rapid suppression of the pituitary that is achieved by antagonists, without an initial stimulatory effect, is the main advantage of these compounds over the agonists. In addition to competitive receptor blockade, other mechanisms of GnRH antagonists action, such as receptor down-regulation, appear to be involved during their long-term administration (Behre et al. 1997).

While the development of GnRH agonists progressed quickly, the antagonists have lagged behind, in part related to their high cost of production. Furthermore, the initial generations of GnRH antagonists were too weak and exhibited side effects, which almost halted their further development (Heber et al. 1982). Specifically, a significant histamine release resulting in anaphylactoid reactions and local solubility limitations adversely affected the widespread usefulness of the first and second generations of antagonists. More recently, a new series of potent, acceptably long-acting (≤ 10 days), water-soluble and low histamine-release third-generation GnRH antagonists were developed (Jiang et al. 2001; Broqua et al. 2002), for example, cetrorelix, abarelix and ganirelix, which are already on the human pharmaceutical market, and antarelix, teverelix, degarelix, ozarelix, ornirelix, azaline B and acyline, which are in clinical trials.

Owing to the peptide nature of the synthetic GnRH analogs, they are susceptible to gastrointestinal peptidase degradation, making oral administration unsuitable. In this aspect, non-peptide orally active GnRH antagonists have been recently developed for humans and may offer a practical alternative for canids and felids in the future (Gobello 2007).

The pharmacological blockade of the effects of GnRH antagonists may be sought for a variety of reasons including assisted reproductive technologies, contraception, treatment of sexual-steroid-dependent disorders (neoplastic or non-neoplastic) as well as the modulation of undesirable sexual behaviour. Additionally, the antagonists can also be used as probes of reproductive processes, allowing a greater depth of understanding of basic endocrinology in a particular species.

The aims of this review are twofold: to compare the effects of GnRH antagonists vs agonists and to describe the existing literature around new antagonists as well as

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their combination with GnRH agonists in domestic carnivores.

New GnRH Antagonists in Domestic Dog and Cat Reproduction

There are limited data concerning the use of GnRH antagonists in domestic carnivores. In dogs, the effect of GnRH antagonists was first described during the 1980s when only the first-generation compounds were available (Vickery 1985; Vickery et al. 1989). More recently, after a 25-year interval, some pharmacokinetic, endocrine and clinical reports appeared using the safer and more potent third-generation antagonists (e.g. cetrorelix, antide and acyline) in domestic dogs and cats (Schwahn et al. 2000; Pelican et al. 2005, 2008; Valiente et al. 2007, 2009a,b,c; García Romero et al. 2009, 2012a,b; Risso et al. 2010).

In a given species, the effects of GnRH antagonists can be described through the complex interactions of the animal gender, the reproductive state and stage, the antagonist's potency and administration protocol (i.e. dose, frequency and duration of treatment) as well as on probably unknown individual factors.

A number of randomized controlled trials were carried out using a single subcutaneous dose of the third-generation GnRH antagonist acyline (Contraception & Reproductive Health Branch Center for Population Research, NIH, Bethesda, MD, USA), and some endocrine and clinical data in domestic dogs and cats are now available and summarized in the different sections below (Valiente et al. 2007, 2009a,b,c; García Romero et al. 2009, 2012a,b; Risso et al. 2010).

Endocrine Effects

In mature male dogs, a single subcutaneous administration of acyline (330 μg/kg) decreased FSH and LH below pre-treatment concentrations 60 min after injection, whereas testosterone (T) diminished to below baseline levels after 90 min without the characteristic surge seen after agonist administration. Then, both gonadotrophins and T diminished until day 9 post-treatment, when they reached their nadir. On day 14 after treatment, the three hormone concentrations gradually began to increase. A clear rebound above baseline could also be seen for FSH and T at the end of the follow-up period of this study on day 29 (García Romero et al. 2009). In a similar complementary study, acyline-treated dogs were serially challenged with the GnRH agonist, buserelin (0.2 μg/kg), over a 30-day period and blood samples for T determinations were collected before and up to 80 min after the agonist injection. On the first 14 days of the experiment, the gonadal axis response to the agonistic stimulation appeared significantly decreased in acyline-treated dogs; from day 21 to the end of the study on day 30, the effect was similar to the placebo group. The conclusion was that in dogs a single administration of the GnRH antagonist reversibly decreased serum gonadotrophins and T concentrations for 9 days and prevented physiological response of the gonadal axis to agonistic challenge during 14 days. In dogs, antagonistic suppression of the pituitary-testicular axis seems to be shorter than that described in humans (Herbst et al. 2004), which may be attributed to pharmacokinetic differences between species.

Testicular and Seminal Effects

The antagonist's protocol described above adversely affected semen quality in male dogs where the volume of the second and third fractions of the ejaculate was also diminished (<0.2 and <0.6 ml, respectively) at the end of the first month after treatment. At the same time, sperm count and total motility presented a clear impairment (<0.5 million/ml and <30%, respectively) with gradual improvement to the end of the study on day 60 after treatment. Morphologically abnormal spermatozoa (e.g. proximal and distal droplets, abnormal and detached heads and coiled tails) increased up to 65% 45 days after acyline administration. There were also increasing numbers of round spermatogenic cells over time, which reflects the gonadotrophin-induced disruption to spermatogenesis (Garrett et al. 2005). Libido and erection were also affected during the first month of the follow-up period. The rapid severe oligozoospermia is difficult to explain; perhaps the low sperm output during the first month after treatment was secondary to the impaired libido. Lack of hormone support may also 1 have severely interfered with sperm maturation in the epididymes.

In mature male cats, the same pharmaceutical protocol significantly and reversibly impaired spermiogenesis, spermatocytogenesis and sperm motility 2 weeks after the antagonist administration as shown by testicular histology and evaluation of sperm recovered from the epididymis tail. In these cats, the histological findings were similar to those described for androgen deprivation in other species (Misro et al. 1992). Furthermore, germ cell development was arrested at the spermatogonia level, which is the major site of disruption of spermatogenesis when gonadotrophin release is suppressed (Garrett et al. 2005). From that cellular level on, there was a significant decrease of all the components of the germinal epithelium up to day 15 after acyline administration, when they progressively returned to pre-treatment histology. The present results in the canine and feline species probably reflect the 2-week suppression of testosterone to baseline concentrations that has been described for new GnRH antagonists in dogs (García Romero et al. 2009, 2012b). 2

Effects on Follicular Phase and Ovulation

In mature, early proestrous (<3 days) bitches, the previously mentioned GnRH antagonist and dose rapidly (3.2 ± 0.2 days) suppressed the progression of the oestrous cycle to ovulation. In these females, vulvar size decreased losing turgidity, and vaginal discharge was diminished to a minimal quantity becoming less haemorrhagic. Furthermore, the animals did not demonstrate typical oestrous behaviour at any time during the trial and ovulation was absent 14 days after treatment, as shown by the finding of basal progesterone (P_4) serum concentrations. Spontaneous return to a normal

oestrous cycle occurred 24.8 ± 2.0 days after acyline. When in another group of bitches, a lower acyline dose (110 μg/kg) was used, a shorter (19.5 ± 2.7 days) postponement occurred, which suggests a dose-dependent effect previously described for antagonists. Although the short cycle interruption obtained in bitches seems to limit the practical use of the third-generation antagonists as contraceptives, the remarkable synchronous return to cycling appears a potentially attractive tool when assisted reproduction techniques are to be applied in this species.

When the same antagonist protocol was administered to early proestrous (<3 days) mature queens, neither the follicular phase nor the following interoestrous intervals were shortened when compared with a placebo group and historical data of the colony. Oestrous behaviour was not affected by treatment (Risso et al. 2010) and all queens were mated with a fertile tomcat, but ovulation and pregnancy did not occur.

The different effects seen with the new GnRH antagonist on follicular phase between the two species were previously reported in different studies in the domestic cat when the third-generation GnRH antagonist antide (two injections of 6 mg/kg 15 days apart) prevented initiation of estradiol surges but failed to curtail oestrogen surges that were already in progress at treatment onset (Pelican et al. 2005, 2008). GnRH antagonist-mediated inhibition of GnRH and gonadotrophin production and release did not seem to affect follicular growth at a certain stage of development. Ovulation inhibition and absence of long-term changes in reproduction following treatment withdrawal have previously been reported (Pelican et al. 2005, 2008; Valiente et al. 2009c; Risso et al. 2010).

Effects on Luteal Phase of the Pregnant Female

When the acyline protocol, cited above, was administered in mid-pregnant (25–35 days from first mating) bitches, gestations were terminated in 6.4 ± 1.3 days and P_4 serum concentrations permanently decreased. The decreasing P_4 rate varied among animals and was closely related to abortion when P_4 reached basal concentrations (Valiente et al. 2009a). The range of days between treatment and abortion could be owing to the variation in gestational ages among bitches or to individual variations to GnRH antagonist response. Assuming that luteolysis was probably due to the GnRH antagonist-induced gonadotrophin suppression, these results would seem to confirm the necessity of LH in maintaining mid-pregnant canine corpus luteum. As GnRH antagonists inhibit not only LH but also FSH, more research is needed in this aspect.

When the same antagonist protocol was used in early, mid and late pregnancy (20–25, 26–45 and >45 days post-mating, respectively) queens, the gestations were maintained and they all gave birth to healthy kittens. In these female cats, P_4 serum concentrations were within normal range for feline pregnancy during the 14 days of follow-up after treatments (Risso et al. 2010). Thus, acyline may not have affected luteal function at any stage of pregnancy. In contrast to the domestic dog (Valiente et al. 2009a), gonadotrophins do not seem to be necessary for corpus luteum support at any stage of gestation in the cat. A similar situation of gonadotrophin independence was also described for the feline non-pregnant luteal phase (Pelican et al. 2008).

GnRH Agonists and Antagonists Combinations

Various combinations of GnRH antagonists and long-acting GnRH agonists have been assessed in several species to prevent the 'flare-up' effect that agonists cause on the pituitary-gonadal axis. In anoestrous females, this initial stimulation induces an undesirable oestrus within the first 2 weeks after agonist administration (Wright et al. 2001). In this regard, when the previous acyline protocol was administered within the first 48 h after the long-term release agonist deslorelin acetate in anoestrous bitches, the initial ovarian stimulation and ovulation were prevented in only a minority of the bitches (Valiente et al. 2009b). It is suggested that stimulation was prevented during the peak antagonistic effect but it was later overridden by the long-term release agonist (Sharma et al. 1992). The pituitary, apparently, remained responsive to agonist stimulation when the antagonist blocking effect waned off. Stage of anestrus at the time of treatment as well as individual variations may also have accounted for the heterogeneous results.

Safety and Reversibility

As expected, this third-generation antagonist did not provoke the systemic allergic side effects reported for earlier compounds (Vickery et al. 1989). Safety was constant finding in all the previously described studies. No animal had haematological serum biochemical (dogs; Valiente et al. 2007), local or systemic (dog and cats) side effects attributed to the acyline treatment. Reversibility of antagonist effects previously described for these compounds (Pelican et al. 2005) was corroborated in most in the above trials.

Discussion and Conclusions

Gonadotrophin-releasing hormone antagonists act by competitive binding to the pituitary GnRH receptors, theoretically offering a more direct treatment alternative. However, GnRH agonists, not antagonists, are currently the primary form of hormone suppression therapy owing to superior administration methods, which include long-acting injections and implants. The main limitation of GnRH antagonist application has been the difficulty in synthesizing long-term release formulations. Thus, until depot formulations were available, the main indication of antagonists appeared to be limited to the management of acute endocrine situations. At present, higher dose rates, serial administrations and non-peptide orally active GnRH antagonists may cause sustained gonadotrophic deprivation, which may increase the utility in the future.

It is concluded that, although GnRH antagonists appear to have a promising future in domestic carnivores reproduction, further pharmaceutical development as well as endocrine and clinical work is

necessary before they can be widely recommended. Finally, care should be taken when extrapolating results to different antagonists, as potency and release rate may differ within a generation.

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Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author contributions

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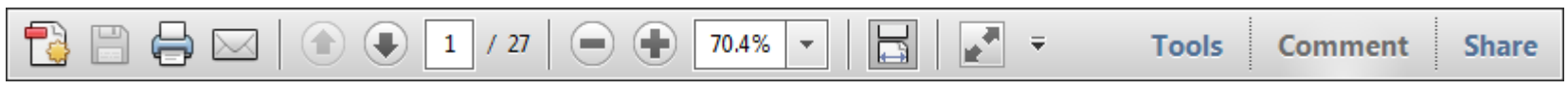

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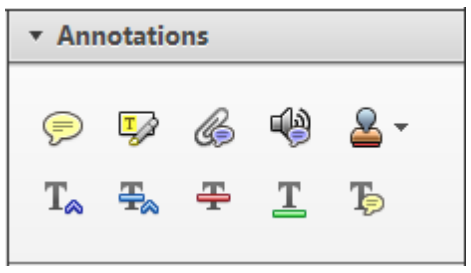

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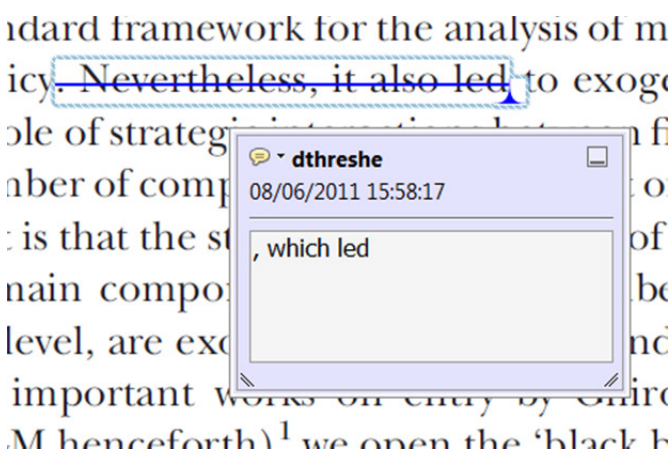

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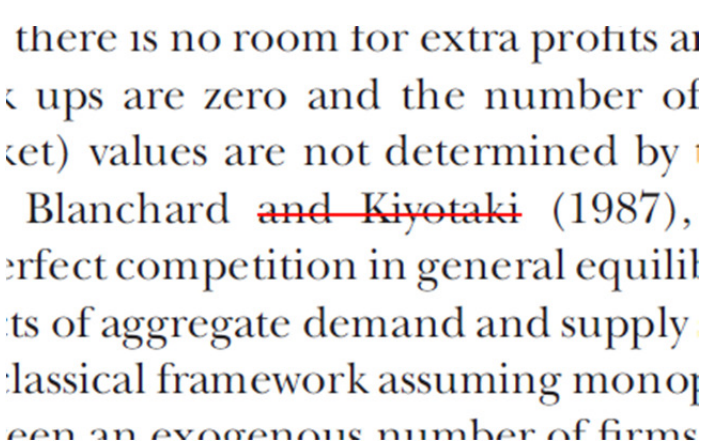

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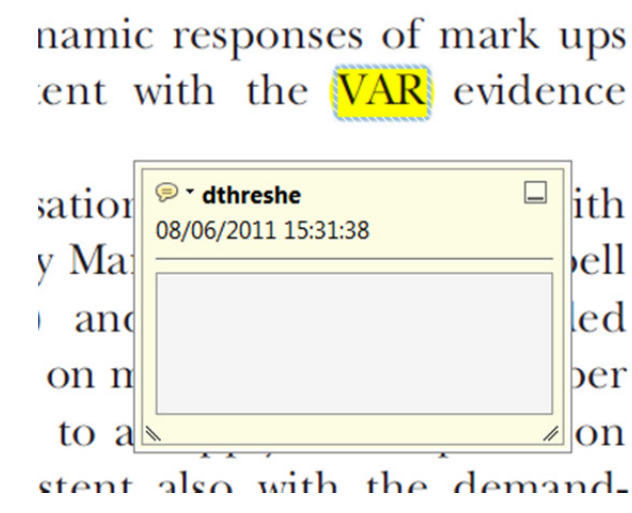

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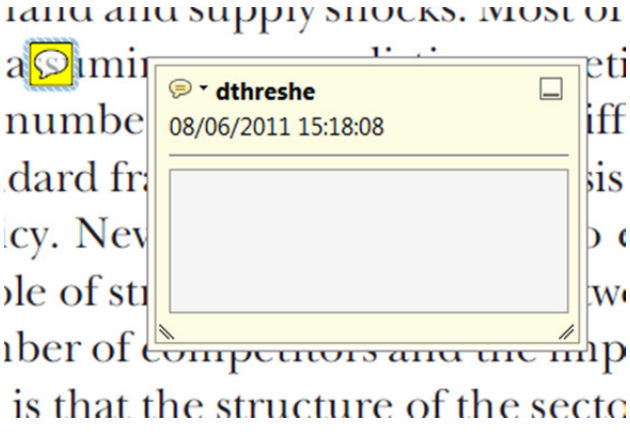

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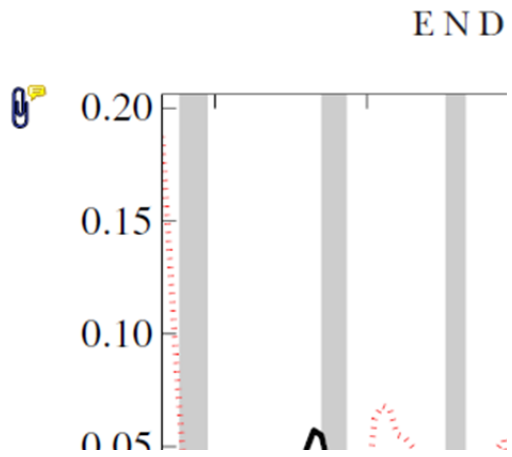

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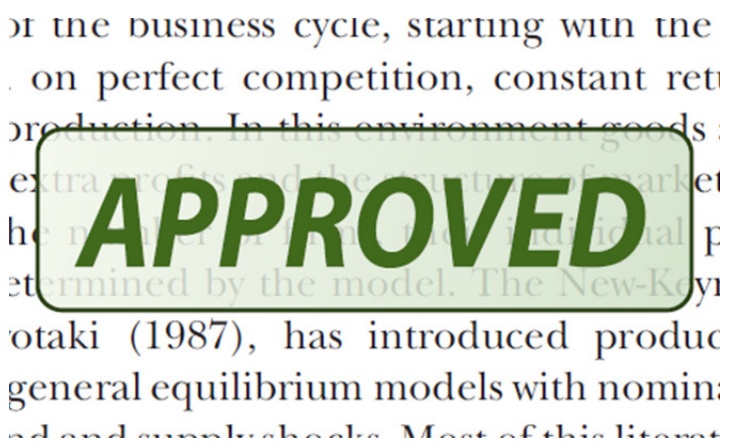

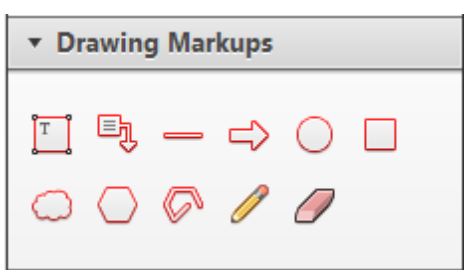

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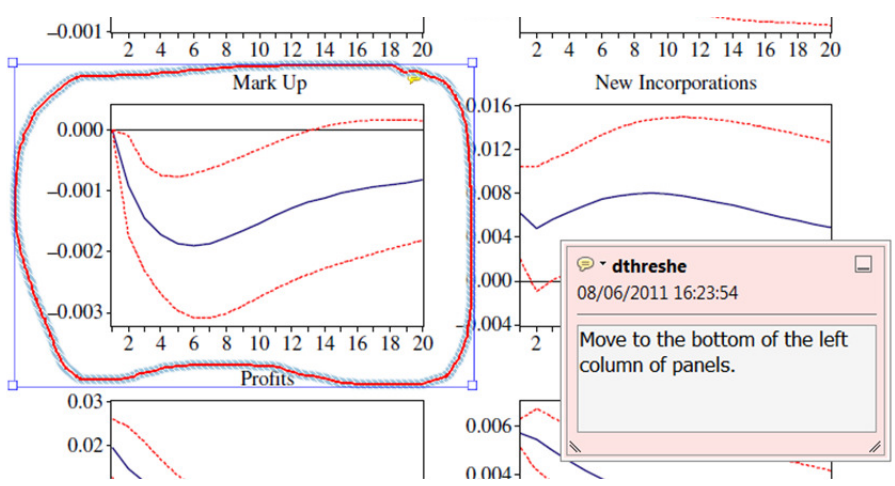
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: