GLEPRISTONE

SCIENTIFIC UPDATE 2010

Summary Table of contents

INTRODUCTION

For many years veterinary practitioners have been called upon to advise and to facilitate the termination of unwanted pregnancies in bitches.

This is a sensitive area of veterinary science often associated with emotional stress for owners and physical distress to bitches. It has also been a notoriously unreliable area of veterinary medicine: products either in current use or that have been used in the area associated with severe side effects, a narrow windows of action, a need for multiple and frequent administrations or a poor efficacy rate.

Today, Aglepristone is a new answer for pregnancy termination in bitches: a highly accurate tool that is at the same time easy to use, effective and safe.

Aglepristone is a progesterone antagonist which is available in an injectable solution.

The potential role of this compound in animal health is huge. It is applicable to a variety of pregnant-related conditions (abortion, induction of parturition, treatment of metritis and pyometra, treatment of certain types of tumor) and to various other therapeutic purposes.

REPRODUCTIVE SYSTEM OF THE BITCH

The reproductive life of the bitch is characterised by a repetitive cycle. The events of this cycle involve sequential changes to the ovaries, sexual organs and behavioural patterns.

The reproductive cycle is also called an "oestrus cycle". This term refers principally to the behavioural state during which the bitch allows mating to occur.

The regulation and coordination of the various physiological and behavioural processes involved in reproduction is the responsibility of the body's endocrine system.

In fact, changes in the frequency and amounts of hormones secreted are responsible for the resultant variation in reproductive function and behaviour.

THE REPRODUCTIVE CYCLE OF THE BITCH

The reproductive cycle begins at puberty and may function throughout the bitch's lifetime, as the bitch does not undergo menopause.

The reproductive cycle (Concannon and al., 1989) consists of two oestrus cycles per year (lasting between 4 - 8 months). The oestrus cycle is in turn subdivided into 4 stages (Figure 1). These stages are defined in terms of the bitch's sexual behaviour. There are breed and individual variations (often size-related) to this pattern.

The 4 behavioural stages are, in sequence:

PRO-OESTRUS ➜ *HEAT*
The average duration is 3-17 days. This stage represents the first obvious evidence of sexual activity. Signs such as a swollen vulva, bloody vaginal discharge and attractiveness to the male are typical but the female refuses mating.

OESTRUS ➜ *HEAT*
The average duration is 3-21 days. This stage is characterised by the acceptance of mating by the bitch. Ovulation and fertilization occur during the course of this stage of reproductive behaviour.

DIESTRUS ➜ *GESTATION OR PSEUDO GESTATION*
The average duration is 2 - 3 months in the non-pregnant bitch. In the pregnant bitch, diestrus lasts 57 days and ends with the litter's birth.

ANESTRUS ➜ *SEXUAL REST*
The average duration is 3 - 10 months. No obvious signs of sexual behaviour are seen. The whelping bitch will produce milk during the first weeks of this stage.

HORMONAL VARIATIONS IN THE BITCH

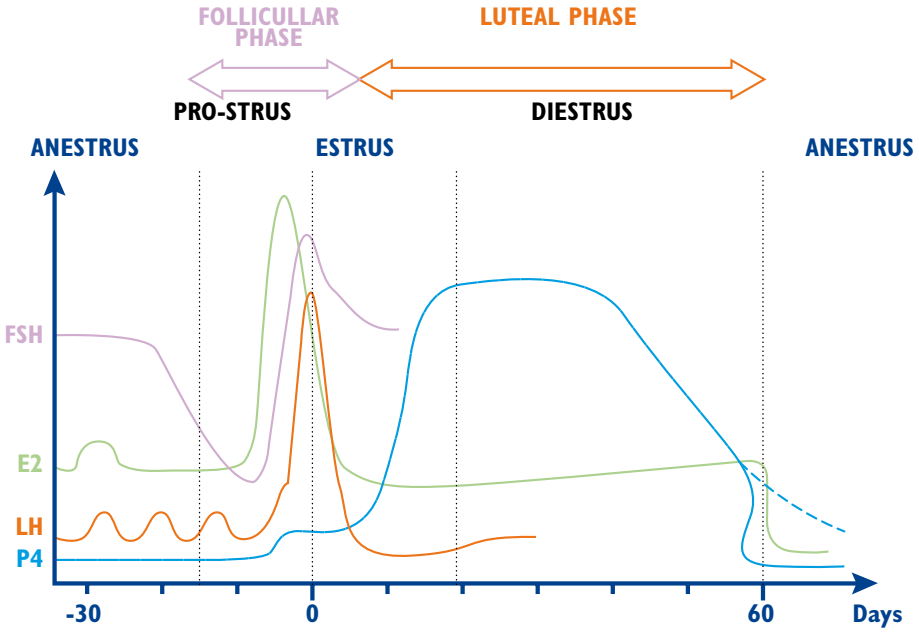


Figure 1: Hormonal variation during the reproductive cycle of the bitch.

Follicular phase

This phase includes the pro-oestrus and the beginning of the oestrus phase.

Follicle growth is stimulated by **Follicle Stimulating Hormone (FSH)**, itself controlled by a surge of gonadotrophin.

These follicles produce **oestradiol (E2)**, which is responsible for the behavioural and anatomical changes seen in pro-oestrus. Oestradiol stimulates thickening of the endometrium, glandular development and synthesis in progesterone receptors, in preparation for pregnancy though progesterone remains at basal level until the end of pro-oestrus.

Preovulatory luteinization and ovulation:
Oestradiol concentration peaks in the last two days of the start of oestrus. Simultaneously follicular cells become luteinised and start producing **progesterone (P4)**, resulting in a rise of progesterone concentration, typical in dog cycle. Increasing progesterone and decreasing oestradiol concentrations stimulate a surge in **Luteinizing Hormone (LH)** followed approximately forty eight hours later by ovulation.

Ovulation leads to the development of corpora lutea.

Luteal Phase

Diestrus is the progesterone dominated phase, which corresponds to the lifespan of the corpora lutea. Progesterone concentrations are elevated during diestrus, peaking around the second or third week of diestrus, paltering and then declining. The decline occurs somewhat sooner and more precipitously in the pregnant than in the nonpregnant bitch due to the onset of the parturition.

Oestradiol concentration remains at basal level during diestrus and does not rise until the last week of the gestation.

The hormonal variations are also dependant on complexes feed-back (Figure 2).

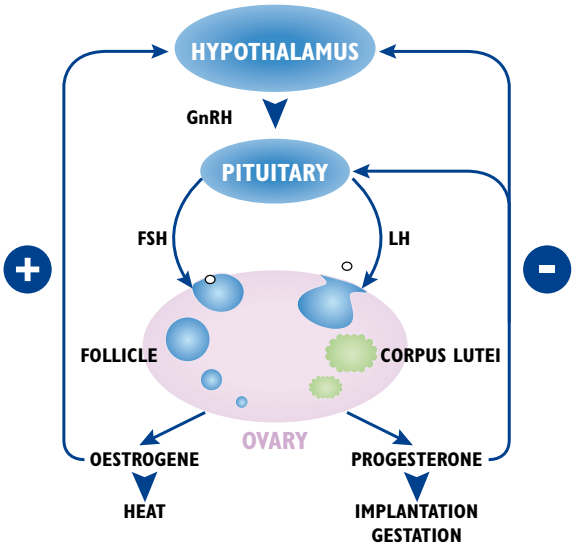


Figure 2: Simplified endocrine physiology of the bitch.

HISTORY OF AGLEPRISTONE

Discovery of mifepristone (R.U 486):

R: as Roussel
U: as Uclaf
486: the product 's code
Mifepristone: generic name (Molecular formula: C29 H35 NO2)

At the beginning of 1975, Georges Teutsch improved the 19-norsteroids-synthesis process at the Roussel Uclaf Research Centre. Although this class of molecules was difficult to synthesise, researchers did not find any interest to look further in fertility control involving this family of compounds. In 1976, G. Belanger, working on the synthesis process of the steroid family, found that these 19-norsteroid molecules had a high affinity for the glucocorticoid receptors.

In 1980, the three following molecules RU 38140, RU 38473 and RU 38486 were considered as a priority by Roussel - Uclaf with the objectives to evaluate the antiglucocorticoid activity. Only one out of these three molecules (RU 486) was able to resist the dexamethasone activity and showed a high affinity for the progesterone receptors. After series of studies on rabbits, RU 486 was considered as an antagonist to progesterone as it was able to bind to the receptors without showing any biological response.

In 1982, Hermann published the first results on the abortive activity of the RU 486 in pregnant women. These results were the start for clinical development of the molecule (Ulman and al., 1986). In 1988, the first marketing authorization for the RU 486 was obtained in human health as an abortive product. When used alone, it has an efficacy rate of 80% and used in combination with prostaglandins, it has an efficacy rate of 99%.

Due to the high pressure exerted in the USA by anti-abortion activists, the product was withdrawn from the market. In France, the Minister of human health decided to maintain the product considering RU 486 to be "women's moral property". In April 1989, RU 486 became available on the French market but only in specialised medical centres. In the USA, Bill Clinton promised women that RU 486 would be available again soon.

In 1994, the American Population Council decided to promote the product but withdrew its decision in April 1997.

Development of aglepristone (R.U. 534):

In 1986, Dr Lavaud was the first veterinarian to be allowed to use RU 486 in a field study on the bitch (Lavaud, 1989) (6 to 23 days after mating).

RU 486 was injected at the dose rate of 5 mg/kg BW, by intra-muscular route for 3 consecutive days. None of the treated bitches had progeny.

Despite the fact that Roussel-Uclaf had obtained an excellent candidate (RU 486), they were still working on a "second best " molecule, more efficient than the previous one regarding the abortive effect and named R.U 534 (Generic name: Aglepristone).

Clinical studies on bitches started in 1992 in the four National Veterinary Schools in France. *The marketing authorization of Aglepristone was obtained in 1996* by Hoechst-Roussel-Vet in France only.

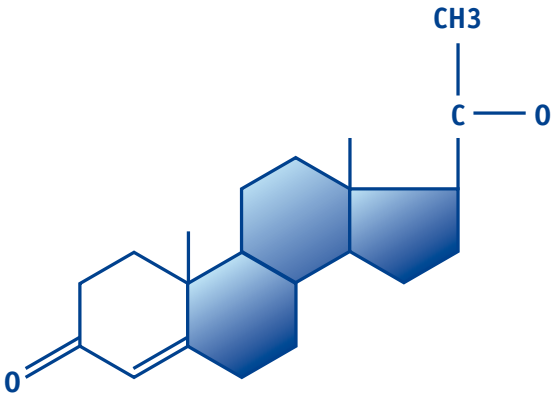
The decision of Roussel-Uclaf to definitively abandon the production of abortive products in both human and animal health, due the boycott threat of activists led this laboratory to sell the license to Virbac Laboratories in 1998. Aglepristone has been distributed by Virbac France SA since October 1998. Since then, a M.A. has been granted for Aglepristone in the following countries:

- Australia in 2000,
- Brazil and Mexico in 2001,
- United Kingdom in 2002,
- and finally it is now available throughout Europe thanks to a mutual recognition procedure in November 2003.

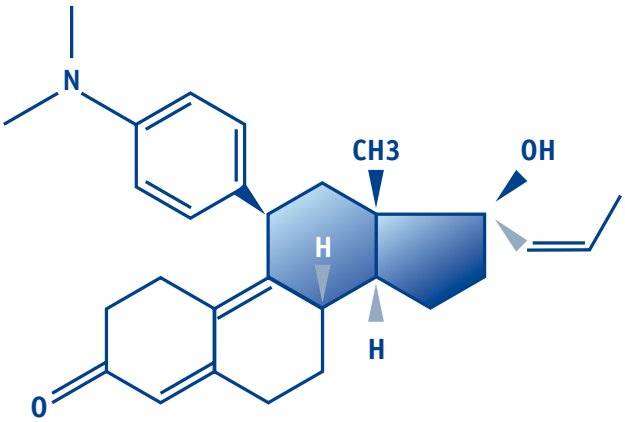
GENERAL CHARACTERISTICS

1.1 PHYSICO-CHEMICAL AND PHARMACEUTICAL PROPERTIES

Generic name:	Aglepristone
Code name:	RU 534
Chemical Abstracts Nomenclature:	(11,17,Z) -11 -[4-(Dimethylamino) phenyl] -17-hydroxy-17- (1-propenyl)-estra-4,9-dien-3-one.
Molecular formula:	C29 H37 NO2
Chemical structure:	Synthetic steroid 19 Norsteroids 11b substitutes derivative from the structure of progesterone (Figure 3)



CHEMICAL STRUCTURE OF PROGESTERONE



CHEMICAL STRUCTURE OF AGLEPRISTONE

Figure 3: Chemical Structures of progesterone and Aglepristone.

1.2 AN ORIGINAL MODE OF ACTION

1.2.1 ANTIPROGESTINS PROPERTIES OF AGLEPRISTONE

Fixation to Progesterone Receptors

Aglepristone is an antagonist which binds to the progesterone receptor, without eliciting the effects of progesterone (no agonist effect) and in doing so prevents progesterone itself from occupying its receptor (Figure 4).

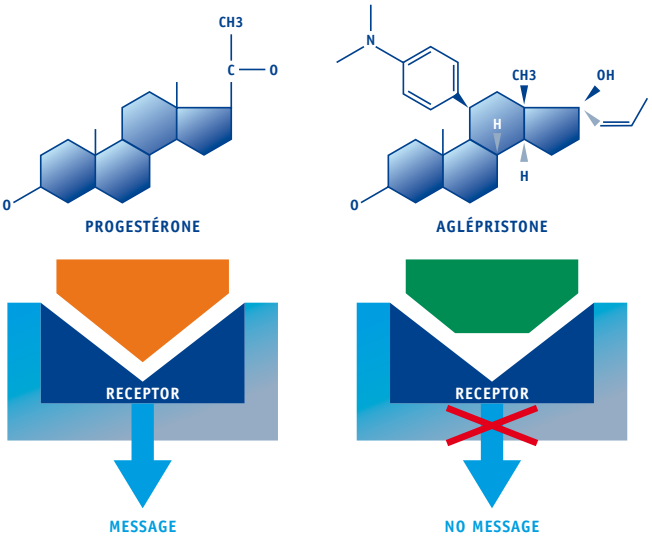


Figure 4: Binding competition between progesterone and Aglepristone for the Progestagen receptors and consequences.

1.2.2 MOLECULAR PHARMACOLOGY

Intracellular mechanism of steroids:

Progesterone, like all steroids, is a small hydrophobic molecule able to diffuse freely through the plasma membrane of cells.
In **target cells**, progesterone (Figure 5)

- ➜ *becomes tightly bound to the receptor and leads to the release of the heat shock protein (HSP)*
- ➜ *the complex of receptor and its hormone moves into the nucleus*
- ➜ *there it will dimerise (association of two receptors)*
- ➜ *and then binds to a progesterone response element (PRE) on the DNA. The progesterone response element is a specific sequence of DNA in the promoters of certain genes that is needed to activate or deactivate those genes*
- ➜ *thus, the complex of progesterone with its receptor forms a transcription factor that binds to PRE, leading to the suppression or activation of transcription and translation of a specific gene sequence regulated by progesterone*
- ➜ *the translation products include structural and secretory proteins, enzymes, and other regulating proteins*

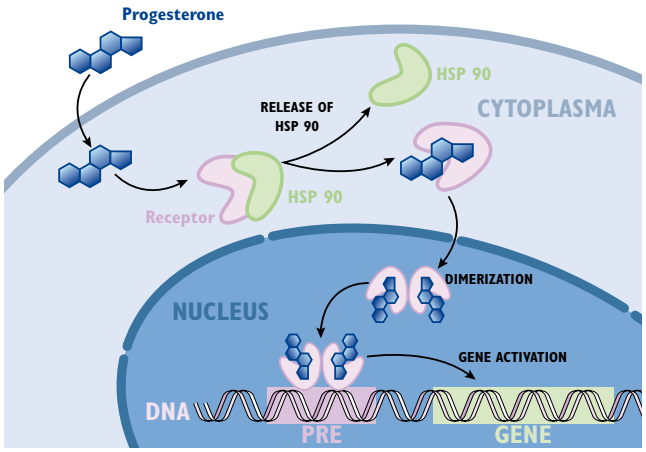


Figure 5: Cellular and molecular mechanism of action of steroid hormone.

1.2.3 ANTIPROGESTATIVE ACTIVITY

Antiprogestins are synthetic steroids, which bind with great affinity to the progesterone receptor without any progesterone effect.

Dr Beaulieu (Beaulieu, 1991) describes for the molecular mode of action of Mifepristone **2 possible mechanisms** to explain the antihormonal effect, which can be obviously transposed:

➜ *RU 534 reinforces receptor-hsp interaction and therefore does not allow the binding of the receptor to the DNA and more particularly to the Progesterone Response Element (PRE) (Figure 6).*

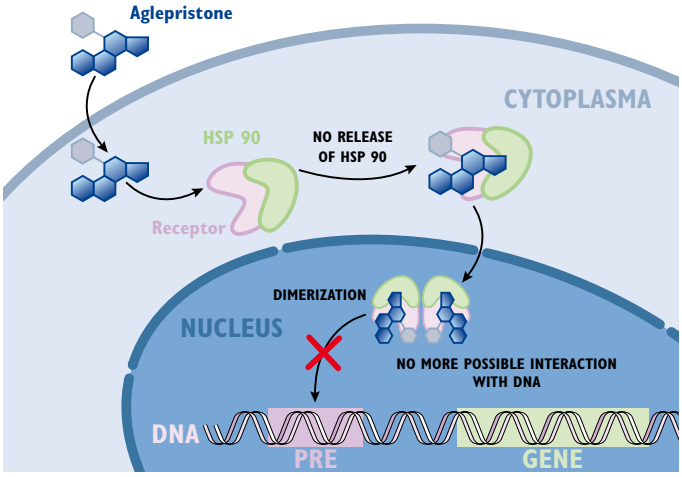


Figure 6: Reinforcement of the receptor-hsp interaction by Aglepristone.

➜ RU 534 binds to the receptor and leads to the release of hsp but the abnormal conformation of the complex inhibits the activation of gene transcription (*Figure* 7).

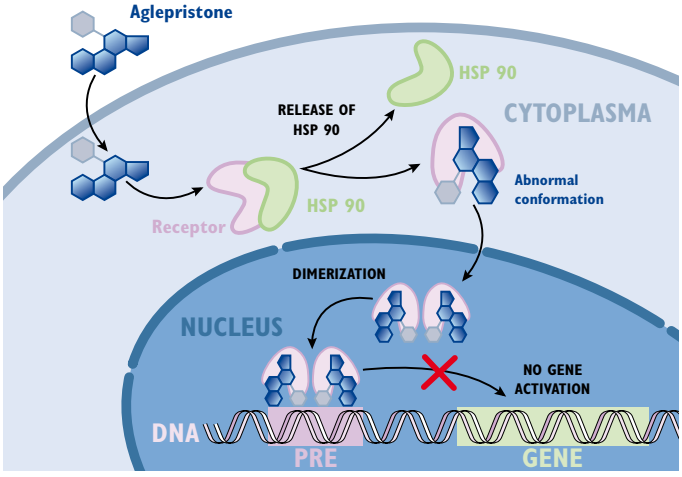


Figure 7: Abnormal conformation of the progesterone-receptor complex.

THUS, THE GENE TRANSCRIPTION NORMALLY ACTIVATED BY PROGESTERONE IS BLOCKED.

1.3 PHARMACOLOGICAL DATA

1.3.1 BINDING AFFINITY (RBA)

To determine *in vitro* affinity of Aglepristone a study was performed with purified uterine Progestagen Receptor (PR) prepared from different species.

It showed that RU 534 has **a very strong relative binding affinity (RBA)** for this uterine progestagen receptor (Figure 8).

Substances	RBA%	
	Dog	Cat
Progesterone 100%	100%	100%
Aglepristone RU 534	312%	926%

Figure 8: Binding competition studies for progestogen receptors, with a reference agonist. By convention, the Relative Binding Affinity of the reference substance is considered equal to 100%.

Another *in vitro* study was performed to determine the RBA for a different type of purified steroid receptors (Figure 9).

The stronger RBA is for the progestagen receptors. There is also a good affinity for the glucocorticoid receptor even if *in vivo* studies did not show any biological effects.

Receptor	RBA (%) of Aglepristone	Reference substance	Result
Progestogen	376%	R5020	Very strong affinity
Glucorticoid	123%	Dexamethasone	Strong affinity
Androgen	8.7%	Testerone	Moderate affinity
Mineral corticoid	0.1%	Adosterone	Negligible affinity
Oestrogen	0.03%	Oestradiol	Negligible affinity

Figure 9: Binding competition study with evaluation of the radiolabeled concentration bound to the receptor. By convention, the RBA of the reference substance is considered equal to 100%.

1.3.2 PHARMACOKINETICS OF AGLEPRISTONE

Plasmatic concentrations follow-up of aglepristone was performed during M.A. Studies (Table 1).

Group	Dose rate	Nb of injection	C_{max} (ng/ml)	T_{max} (day)	MRT (day)
1	20 mg/kg	1	284.5	2.2	5.7
2	20 mg/kg	2 inj. of 10 mg/kg/d at 24-hour interval	287.3	2.7	6.2
3	15 mg/kg	3 inj. of 5 mg/kg/d at 24-hour interval	210	3.2	5.9
4	5 mg/kg	1	67.3	1.7	4.5

Table 1: Determination of the pharmacokinetic parameters (Cmax, Tmax, MRT) and relation between pharmacokinetics and therapeutic effects.

After 2 injections of 10 mg/kg/day at 24 hoursour-interval, the peak plasma concentration (Cmax) is about 280 ng/ml. This peak is reached within 2.7 days (Tmax). The Mean Residence Time (MRT) is about 6 days, which includes the mean absorption time from the injection site.

The results showed that aglepristone follows a linear kinetic pattern because the concentration peak (Cmax observed) is proportional to the dose and appears in a time period (Tmax observed) independent of the dose after one administration.

After administration at a dose rate of 10 mg/kg, **excretion is very slow**. Only 60% of the administered dose is excreted during the first 10 days and about 80% over 24 days.

Excretion is essentially via the faeces (about 90%) with the urine route remaining minor (7%).
This corresponds to what we know about excretion of steroid hormones. The slowness of excretion is explained by the marked lipophilia and the almost certain existence of an entero-hepatic cycle.

1.4 GENERAL SAFETY DATA

1.4.1 SINGLE OVERDOSE TOXICITY

Overdoses studies performed in mice and rats by Oral or Subcutaneous administration show no signs of major toxicity.

➜ LD_{50} *Oral route > 2000 mg/kg*
➜ LD_{50} *Sc route > 1000 mg/kg*

1.4.2 TOLERANCE SPECIFIC STUDIES

Local reaction was observed with increasing dose, incl. erythema, oedema, slight enlargement of inguinal lymph nodes ± ulceration, resolving within a few weeks.

But *no variation* was observed in
➜ *haematological + blood biochemical dosages*
➜ *hormonal dosages (progesterone, prolactin, cortisone and oestradiol)*
➜ *vaginal smears*
➜ *or urinary dosages*

1.4.3 MUTAGENICITY

Several mid- and long-term tests have shown that aglepristone lacks any mutagenic property.

GENERAL CHARACTERISTICS CONCLUSION

Injectable solution

Antagonist of progesterone receptor

Affinity 3 times more important than progesterone

Better tolerated in SC route

No signs of Major Toxicity

No Major Side Effects except a slight local reaction

CANINE PREGNANCY TERMINATION

INTRODUCTION

Although nowadays most bitches are kept closely under control by their owners, pregnancy termination may still be proposed in different situations:

- ➜ *Mismating: i.e. a pure bred female mated with a male belonging to another breed or with a mongrel dog;*
- ➜ *Age: a young bitch at her first heat when the pelvis development is not completed, an old bitch after 8 to 9 years of age (higher risk of dystocia or hypogalactiae);*
- ➜ *High risk of dystocia or cesarean-section: vaginal abnormalities, pelvis fracture or infantilism, single pup syndrome in large or giant breed…*
- ➜ *Medical indication: hormonal disease (diabetes), metabolic problem (pregnancy ketosis) or any disease that may be increased by pregnancy (renal failure, cardiac insufficiency).*

A medical approach is most of time proposed and several protocols are described in the literature (Table 2), but *the use of progesterone receptors blockers at the uterine level tends to become the standard in Europe*.

2.1 PREGNANCY TERMINATION IN THE BITCH

2.1.1 CANINE PREGNANCY TERMINATION BY PREVENTING IMPLANTATION: USE OF ESTROGENS

For years, veterinarians have used estrogens to terminate unwanted canine pregnancies. Estrogens work by preventing implantation. The first injection has to be administered within 4 to 7 days following mating and 95% efficiency is reported (Sutton, 1995). However, it is not suitable for the demands of owners who do not know the precise date of mating. Moreover, many secondary effects may occur: oestrus prolongation, medullar hypoplasia or aplasia, further sterility due to sclerosing effect on the pituitary cells secreting gonadotrophins. Giving estrogens during the beginning of the luteal phase may predispose bitches to develop pyometra (Whitehead, 2008).

Therefore, for safety reasons, estrogens are no longer recommended as a pregnancy termination method in dogs.

2.1.2 CANINE PREGNANCY TERMINATION: INTERFERING WITH PROGESTERONE IMPREGNATION

Progesterone is secreted by the *corpus luteum* in bitches and is maintained at a high level throughout pregnancy (Concannon and al., 1989). Thus, this hormone is responsible for different effects and stimulation of the development, which all contribute to the maintenance of pregnancy (Figure 10):

- ➜ *Stimulation of the development, differentiation and glandular secretion of the endometrium of the pregnant uterus*
- ➜ *Stimulation of endometrial secretion of specific compounds required for:*
  - *pre-implantation embryo development*
  - *embryo attachment*
  - *nidation*
- ➜ *Reduction of myometrial contractility*
- ➜ *Closing of cervix*
- ➜ *Support of placenta formation and maintenance of placental attachment and maintenance of uterine quiescence*

Therefore, interfering with progesterone impregnation can lead to pregnancy termination. This can be achieved by inducing luteolysis or by mimicking the absence of this hormone using antiprogestagens.

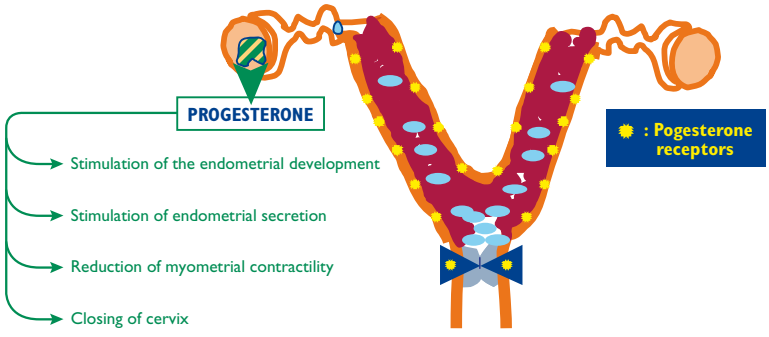


Figure 10 : Action of progesterone on the uterus.

2.1.2.1 Inducing luteolysis with prostaglandins F2α (PGF2α)

PGF2α acts on pregnancy termination through two main effects:

➜ *A luteolytic action, which is the essential one for pregnancy arrest*

➜ *An uterotonic action, which, together with a cervical dilatation following the drop of progesterone, allows the expulsion of intra-uterine fetal debris*

These drugs are mainly used in mid-pregnancy (after days 25 - 28) (Concannon and Hansel, 1977).

PGF2α possess short acting and *dose dependent secondary effects* (Fieni and al., 1997). Gastrointestinal effects are more frequent: hypersalivation, emesis, reflex defecation and/or urination. Some authors do *not recommend* their use in bitches suffering from cardiac or pulmonary dysfunction or in brachycephalic breeds (Romagnoli and al., 1991).

2.1.2.2 Inducing luteolysis with antiprolactins

In dogs, after 30 days of pregnancy, prolactin becomes an absolute requirement for luteal function. Therefore, during the second half of pregnancy in the bitch, antiprolactins like cabergoline or metergoline possess a luteolytic action. In veterinary practice they are often used as co-abortive agents together with other drugs (PGF2α) (Corrada and al., 2006). Very good results were obtained, as 92 - 93% efficiency was recorded.

When used for pregnancy termination, *these drugs may induce emesis or restlessness*.

2.1.2.3 Use of corticosteroids

How corticosteroids induce pregnancy termination is poorly understood. It may possess a direct anti-progesterone effect. Some authors suggest that it may upregulate the uterine and placental prostaglandins synthesis, thus leading more or less to the same events as those which precede normal parturition (Wanke and al., 2006).

Corticosteroids must be used after 30 days of pregnancy. Dexamethasone is the most widely used molecule and pregnancy termination occurs between 7 to 13 days after the start of treatment.

However, they carry numerous side-effects: a brownish vulvar discharge may be observed for several days, making the owner of the bitch fear pyometra, which is in fact not the case. Prolonged polyuropolydipsia may occur, so does a transient weakness possibly due to a transient adrenal suppression, lactation or the risk of live fetuses being aborted.

Due to these side effects, corticosteroids are not recommended for pregnancy termination.

2.1.2.4 Mimicking the absence of progesterone with antiprogestagens

Antiprogestins are synthetic steroids which compete for endogenous progesterone for binding with a greater affinity to progesterone receptors, preventing progesterone from exerting its biological effects (Hoffmann and Schuler, 2000). They act as *true receptors antagonists at the uterine level*. By the way, they prevent the uterine effects of progesterone without initially decreasing serum progesterone concentrations. Used in early pregnancy, it will lead to death and subsequently to embryonic resorption, whereas in mid pregnancy, it will lead to subsequent expulsion of the fetuses.

2.1.2.4.1 Use of mifepristone (RU486)

The first studies for pregnancy termination in the bitch were conducted in the late 80's using the human drug mifepristone and 75% efficiency was reported (Sankai and al., 1991). Oral administration of mifepristone during 5 days from the 32th day of pregnancy successfully induced abortion in all treated bitches 3,5 to 4,5 days after beginning of the treatment (Concannon and al., 1990). No side effect was reported in any of these studies.

2.1.2.4.2 Use of aglepristone (Alizin®)

Nowadays, the antiprogesterone licensed drug in most European countries is aglepristone (Alizin®). This compound is similar to mifepristone, but has less glucocorticoid action and is available as an injectable form, which makes it easier to use. *Aglepristone can be administered at any time during the luteal phase*. Most of the protocols are describing its use in early pregnancy (from day 0 to day 22 of pregnancy, before signs of pregnancy have been established) or until the end of midpregnancy (day 45 of pregnancy, which will lead to the expulsion of the foetuses), but its use in late pregnancy (after day 45, off-label use) is also described in association with prostaglandins. Several studies were realized on its abortive action and are reviewed in the second part of this chapter.

	Antiprogestin		Estrogens	Corticoids	Prostaglandins	Prolactin inhibitors
	Aglepristone	**Mifepristone**	**-**	**Dexamethasone**	**Cloprostenol**	**Cabergoline**
Efficacy	Excellent (99%)	Good (75%)	Average	Satisfactory	Good (79 - 92%)	Satisfactory
			Protocol			
Administration mode	Injection (SC)	Oral	Injection (SC)	Injection (SC)	Injection (SC)	Oral
Dose	10 mg/kg	10 - 20 mg/kg	10 µg/kg	0,2 mg/kg	2.5 µg/kg	5 ug/kg
Repetition	Twice	Everyday	Every 48 hours	Everyday	Every 48 hours	everyday
Duration	2 days	5 days	3 injections	7 to 14 days	> 6-day treatment (3 or 6 injections)	for up to 28 days
Windows administration	From D0 to D45	From D0 to D45	From D0 to D4	From D30	From D35 to 45	From D22 to 45
Side effects	Inflammatory local reaction (spontaneously resolved) Light serous vulvar discharge	No side effects reported	Myelosuppression Cystic endometrial Hyperplasia Cystic lesions of the cervix Pyometra	Brownish discharge Polyuropolydypsia Weakness Adrenal suppression	Salivation, Nausea, Vomiting Abdominal pain Diarrhea Pyrexia Tachycardia, Dyspnea	Nausea, Vomiting Apathy Anorexia Polydipsia Bleeding

Table 2: Comparison of different molecule for canine abortion.

2.2 CANINE PREGNANCY TERMINATION USING AGLEPRISTONE

2.2.1 OFFICIAL PROTOCOL FOR CANINE PREGNANCY TERMINATION USING AGLEPRISTONE

- If the bitch was mated between 0 and 45 days, Aglepristone is a treatment of choice
- If the animal is on heat
  - *advise the owner to avoid further matings following treatment*
  - *or wait until the end of the heat period to administer the aglepristone treatment*
- The animal has *to be weighed* for an accurate dosage
- *0.33 ml aglepristone/kg/day* (i.e. 10 mg aglepristone/kg/day) must be administered
- In 2 Subcutaneous injections *24 hours* apart, under strict aseptic conditions
- At the *cervico-thoracic junction level*
- After the administration a *light massage* at the injection site is recommended, in order to reduce local reaction. Change sides of injections site.
- Furthermore, doses *greater than 5 ml* have to be administered in separate sites
- Plan a repeat visit with the owner to follow up the treatment (and to confirm termination of pregnancy by ultrasonographic examination):
  - *In early pregnancy termination*: 20 days after treatment
  - *In mid pregnancy termination*: 8 days after treatment
- In case of failure (less than 5% of cases), it is possible to repeat the treatment (2 injections of 0.33 ml aglepristone/kg/day 24 hours apart) if the pregnancy has not reached more than 45 days.

2.2.2 CLINICAL RESULTS

Two main studies (support of the registration dossier) can be found in the literature concerning the efficiency of aglepristone alone to successfully induce canine pregnancy termination:
Study 1: conducted in the four French veterinary schools (n=104 bitches) (Fieni and al., 1996)
Study 2: conducted in general practitioners clinics (n=263 bitches) (Fieni and al., 2001a)

2.2.2.1 Protocol

These studies had the same treatment regimen and protocol design as in figure 11.

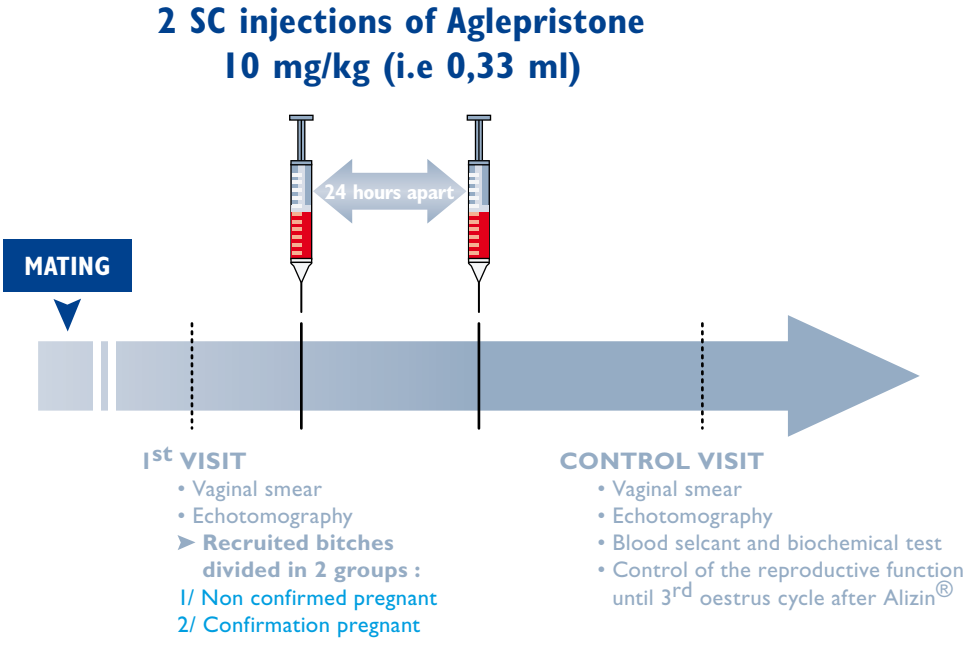


Figure 11: Global protocol design.

Two groups received the treatment:
One with non confirmed pregnancy: 35 bitches which had been mated 10.5 days before in average (Fieni and al., 1996).
The second with confirmed pregnancy: 69 bitches with a mean gestation length of 35.3 days (Fieni and al., 1996).

2.2.2.2. Results

Early pregnancy termination was achieved in 100% cases in the two studies, but the accurate efficacy level should be slightly lower, ranging in 95% confidence interval, i.e [97.4% - 100%] as determined from the results of study 2 (Figure 12 and 13).
Efficacy was lower in mid pregnancy termination. However, *success rates remained at very high level* in the 2 studies, i.e *higher than 94%* with a 95% confidence interval of [88.7% - 97.7%] as determined from the results of Study 2.
In all cases uterine vacuity was observed 1 to 7 days after beginning of treatment and the median value for abortion was 4 days (Fieni and al., 1996) (Figure 12).

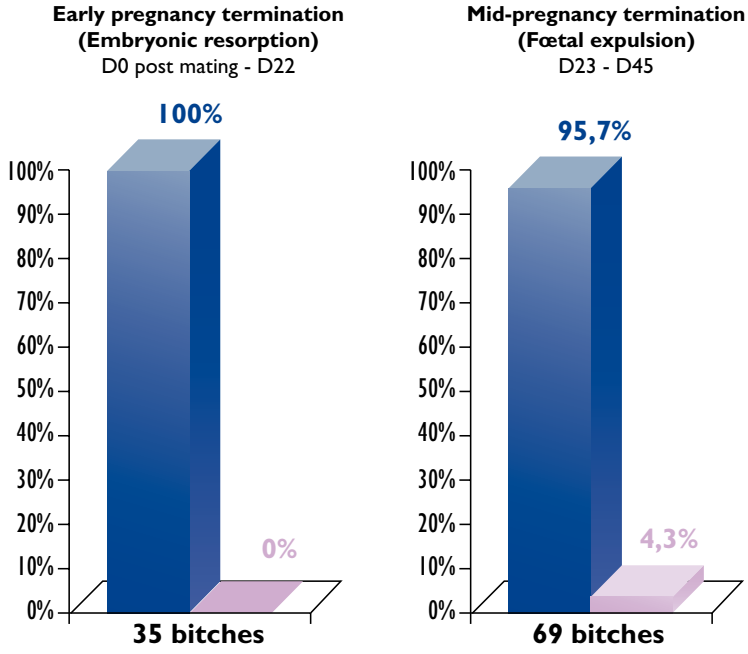


Figure 12: Abortion rate after treatment (Fieni and al., 1996).

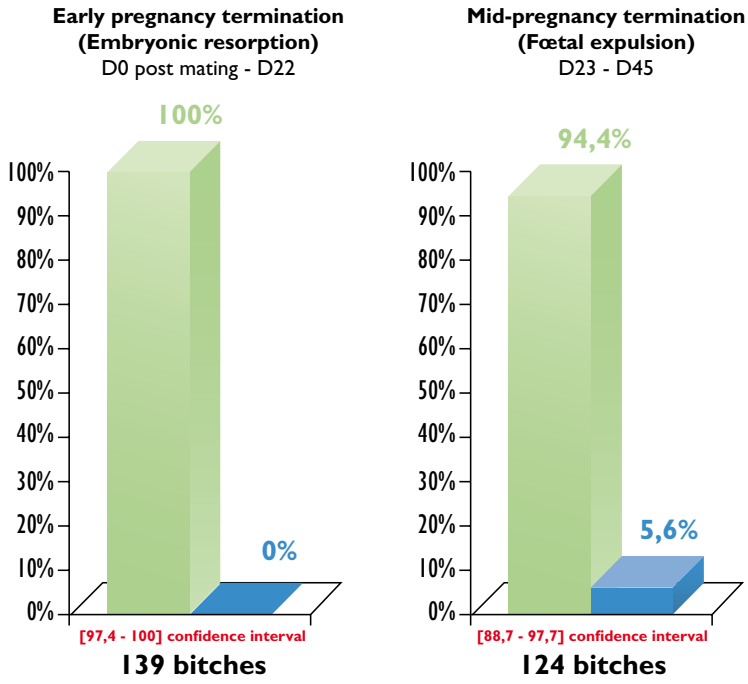


Figure 13: Abortion rate after treatment (Fieni and al., 2001a).

2.2.2.3. Other reports from the literature

The same protocol was applied in several studies (Galac and al., 2000; Corrada and al., 2005; Schäfer-Somi and al., 2007; Kanca and al., 2008) and *efficacy was reported between 95 to 100%.*

Petterson and Tidholm (2009) used one injection of 20 mg/kg SC to induce abortion in the bitches and they reported 95% efficacy. The authors did not mention an increase in the number of local reactions with the use of this protocol.

Pregnancy termination occured *4 to 11 days after beginning* of treatment (Galac and al., 2000; Corrada and al., 2005; Schäfer-Somi and al., 2007), which is shorter than what is observed when using prostaglandins alone (Fieni and al., 1996; Galac and al., 2000).

Combining aglepristone and PGF2α was reported (Hoffmann and Schuler, 2000). Aglepristone was used at 10 mg/kg twice 24 hours apart, and 24 hours later cloprostenol was injected at 1.5 µg/kg twice 24 hours apart. *Combining the two compounds could provide a way to induce abortion in late pregnancy* (after 45 days), as it will decrease the side effects related to prostaglandins and allow expulsion of the foetuses. However, bitches undergoing this treatment should always be hospitalized, as the expulsion of foetuses after 45 days could affect most of the owners.

2.2.3 LACK OF EFFICACY

The pharmacovigilance survey after use on large scale confirms the *excellent efficacy of aglepristone*, as failure cases represent only 0.11% out of the treatments performed.

Partial abortion with remaining dead or living puppies, or a normal pregnancy led to full term were observed. Complications of failure (placental retention, uterine rupture, peritonitis and advanced return to oestrus) were observed.

The incidence of failures has dropped over the last few years since more and more practitioners now monitor the efficacy of treatment by an ultrasonographic examination of the uterine vacuity, and know that, in case abortion does not occur, they can possibly give a second treatment up to day 45.

No failure after two successive treatments was reported.

2.2.4 EFFECTS OF THE TREATMENT

2.2.4.1 General signs

Fieni and al. reported that the use of aglepristone in early pregnancy will lead to *embryonic resorption*, whereas its use in mid pregnancy would lead to *abortion*. However, aglepristone was proved to be efficient at any stage of pregnancy (Fieni and al., 2001).

➜ In early pregnancy termination:

- In 8 - 12% of females, a *light serous discharge* can be observed, but most of time, no obvious signs of pregnancy termination will be noticed by the owners.

➜ In mid pregnancy termination:

- If the early pregnancy administration leads to an embryonic resorption, the mid pregnancy termination ends in a foetal expulsion less than seven days for 95.7% of cases studied.

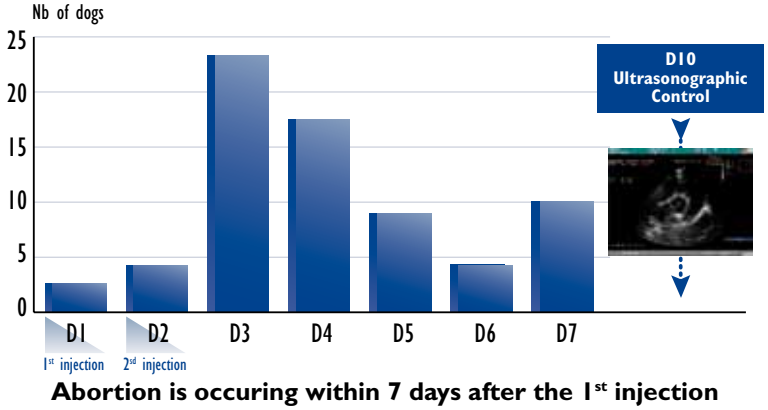


Figure 14: Distribution of the time period for obtaining abortion in mid pregnancy termination.

Fieni and al. (1996) reported that 33% of bitches in mid pregnancy presented a *brownish vulvar discharge* 72 to 96 hours after initiation of the treatment (Figure 14).

Hypothermia, slight depression and transitory anorexia may appear during the abortion itself. These symptoms correspond to a *physiological phenomenon inherent to parturition* and therefore are negligible especially when compared to the clinical signs observed during abortion induced by prolactin or prostaglandins.

5.7% of the bitches treated with aglepristone developed a pyometra (Figure 15). This rate is much lower that what was reported when using other abortifacients: for instance, 10 - 15% bitches will develop pyometra after abortion using estrogens. There are few data concerning the natural incidence of spontaneous genital complications after oestrus in the bitch.
According to studies performed on an animal insurance database containing data over 200,000 dogs in Sweden, the rate of uterine complications was around 2% in 1995 and 1.9% in 1996 (Egenvall and al., 2001). According to another study performed in Japan, incidence of uterine disease was around 15% in a beagle colony (Fukuda, 2001). Therefore, *there may not be any direct relation between the treatment and the development of uterine diseases*.

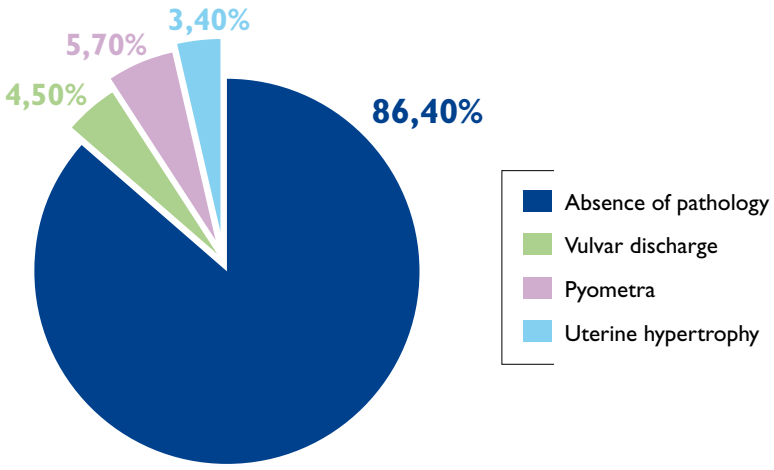


Figure 15: Incidence of post abortive diseases (Fieni and al., 1996).

In almost all the studies, a *pain reaction and a local intolerance* more or less pronounced and dependent on the injected volume were observed at the injection site. Most of time, these reactions *spontaneously resolved*.

Local complication such as cutaneous necrosis, swelling and abscesses may develop as a consequence of scratching or licking at the injection site.

2.2.4.2 Effect on the reproductive function

Aglepristone has no consequence on the reproductive function except a *shortening of the interoestrus interval*. The delay of return to oestrus after pregnancy termination may be reduced from 1 to 3 months. This may be linked to a central effect of aglepristone at the hypothalamo-pituitary level, but this remained to be ascertained (Galac and al., 2000; Galac and al., 2004).

The *fertility rate* after abortion in the first oestrus is 86.2% but returns to 100% after the second oestrus. It is recommended to wait for the second heat if the bitch has a high reproductive value.

After treatment, heats proceed physiologically and ensure *normal pregnancies and parturitions*.

According to Fieni and al., (1996), where bitches received from 0 to 135 mg/kg of aglepristone, the abortion *repercussions of aglepristone on subsequent litters are minimal* since they are limited to a slight increase in the size of the litter following the treatment.

The health of the puppies was not affected at all by previous administration of aglepristone.

AGLEPRISTONE DOES NOT IMPAIR REPRODUCTIVE FUNCTION. NO INFERTILITY CASE OCCURS AFTER ADMINISTRATION.

2.2.4.3 Hormonal changes following the use of antiprogesteron compounds

The use of antiprogestagen compounds allows pregnancy termination while progesterone plasmatic values remain at a high level (Galac and al., 2000; Fieni and al., 2001).

Administration in early pregnancy (D12 after mating) did not lead to any hormonal modifications. Progesterone and prolactin rates that were observed were similar to non treated bitches at the same stage (Galac and al., 2004).This could be linked to a central effect on the hypothalamus, which could be refractory to the treatment at this period and inhibition of progesterone receptors with aglepristone would not lead to an excessive stimulation.

The situation is rather different in *mid pregnancy*. Surprisingly, the application of aglepristone first leads to an increase in *progesterone plasmatic levels* during the first 24 hours (Schäfer-Somi and al., 2007), followed by a slight decrease (Galac and al., 2004). An increase in the *plasmatic levels of prolactin* is observed 24 hours after, as it usually occurs in normal parturition (Galac and al., 2000; Fieni and al., 2001). An increase in the *plasmatic level of prostaglandins* is also observed, which could be explained by a direct effect at the uterine level, as demonstrated with mifepristone (Spitz and Bardin, 1993).

Therefore, when aglepristone is administered, the endometrium mimics an absence of progesterone and its metabolism is not stimulated enough, what will lead to *embryonic death and resorption or expulsion of the fetuses*. The following luteolysis would be linked to secretion of prostaglandins by the uterus.

2.2.4.4 Haematological and biochemical signs

A tendency towards absolute hyperglobulinemia may be observed in some bitches. It is also possible to observe some changes in enzyme activity:
➜ ALT (Aspartate aminotransferase) may increase 14 days after the injection of aglepristone
➜ ALP (Alkaline phosphatase) may also vary.

Analyses performed showed *no biological sign of toxic effect of aglepristone on bone marrow or on the kidney or the liver metabolism.*

AGLEPRISTONE DOES NOT ALTER EITHER HAEMATOLOGICAL OR BIOLOGICAL PARAMETERS.

CANINE PREGNANCY TERMINATION CONCLUSION

Easy to use

Very effective

No *toxic effect* on bone marrow, kidney or liver functions

No consequential effects on the future *reproductive function*

A treatment of choice for pregnancy termination in canine.

FELINE PREGNANCY TERMINATION

INTRODUCTION

Pregnancy termination is less frequently requested for queens, as most of the young queens are neutered around the age of puberty. When they are not, most of time mating will not be noticed by the owners and pregnancy will only be suspected only when an abdominal enlargement is observed, generally in mid pregnancy. If some owners will wait for parturition, the others will ask their veterinarians to perform an ovariohysterectomy to remove the kittens, and very few of them will ask for a medical abortion.

Nowadays, aglepristone is a well known drug used for pregnancy termination in bitches with satisfactory efficacy and excellent general safety. Even if this application is less documented in queens, its use can be considered.

3.1. OTHER ABORTIFACIENTS USED IN THE QUEEN

Protocols that can be used in the queen are very similar to the ones we previously described in the bitch, as these two species share the same physiology of pregnancy (Axner, 2008). As this indication is rarely requested by the owners, very few studies had been carried out on the topic, but the use of estrogens, prostaglandins F2α and antiprolactins like cabergoline are described (Shille, 1982; Concannon and al., 1990; Eilts, 2002).

Estrogens have to be administered during the very following days after mating, which makes their use inconvenient for medical abortion in the queen. Moreover, the same disadvantages as in dogs are described: lengthening of estrus, uterine infection, risk of sterility. Thus, their use is no more recommended.

Prostaglandins and antiprolactins can be used in mid pregnancy and proved to be efficient. However, they have to be administered during several days and side effects can be exhibited by the treated animals as described in the dog.

Therefore, aglepristone can be considered as an interesting alternative in this indication.

3.2. FELINE PREGNANCY TERMINATION USING AGLEPRISTONE

3.2.1 PROTOCOLS DESCRIBED IN THE LITERATURE

In queens, the progesterone receptors *in vitro* have a higher affinity to aglepristone than in bitches (3 times higher than the one in the natural hormone for bitches, 9 times higher in cats). Nevertheless, *for kinetics reaction reasons, higher doses must be used (15 mg/kg)*.

Several studies concerning the use of aglepristone as an abortifacient in the queen can be found in the literature (Fieni and al., 2006; Georgiev and Wehrend, 2006; Georgiev and al., 2008; Goericke-Pesch and al., 2010) and the protocols that were used were similar to what is described in the bitch. Aglepristone was injected *twice subcutaneously* at the posology of 10 mg/kg (Georgiev and Wehrend, 2006; Georgiev and al., 2008; Goericke-Pesch and al., 2010) or 15 mg/kg (Fieni and al., 2006) 24 hours apart, 24 to 55 days after mating.

A control of the uterine vacuity one week after the 1st injection is recommended, as in the bitch.

Abortion was successfully induced in queens even on day 55 of pregnancy. However, from an ethical point of view, it is not recommended to treat later than day 50 as there is a greater risk for kittens to be born alive, which can affect the owner. If this has to be done, hospitalization should be proposed.

3.2.2 CLINICAL RESULTS

3.2.2.1 Use for early pregnancy termination

A recent study (Goericke-Pesch and al., 2010) has tested the efficacy of aglepristone for prevention of early pregnancy in cats. Cats were treated with 10 mg/kg aglepristone on day 5 and 6 after mating. Another group of cats were used as control. None of the queens that were treated was pregnant at an ultrasonographic examination on day 25 (whereas 88% of the untreated queens were pregnant). *No major treatment-related side effects* were observed and *pregnancy rates* following treatment were 64 and 82% respectively after the first and the second estrus respectively.

3.2.2.2. Use for mid to late pregnancy termination

When used at 15 mg/kg, the *efficacy of aglepristone was 88.5%* and termination of pregnancy was achieved in 50% of the queens within 3 days (Fieni and al., 2006).

When used at 10 mg/kg, the treatment successfully induced abortion in 87% of the treated queens and the mean duration of abortion, defined as time span from first occurrence of vaginal discharge to expulsion of all fetuses observed by ultrasonographic examination was 1 day in 9 cats, 2 days in 5 cats and less than 1 day in five cats (Georgiev and Wehrend, 2006).

Therefore, the use of a dose of 10 mg/kg seems efficient enough to successfully induce abortion in mid pregnancy in queens.

Georgiev and al. (2008) studied the histological modifications affecting the cervix, endometrium and placenta following mid pregnancy termination with aglepristone in the queen. Abortion of queens through aglepristone given during mid gestation is assumed to be the result of damages of uterine veinules which lead to an interstitial haemorrhages and bleeding into the uterine lumen, subsequently resulting in *utero-placental detachment*.

3.2.3 EFFECTS OF THE TREATMENT

3.2.3.1. Clinical signs reported

Brief periods of depression and anorexia were reported in 9.3% of the queens before foetal expulsion and these symptoms could be attributed to the phenomenon of abortion (Fieni and al., 2006).
Brown mucoïd serous discharge due to placental detachment occurred in 59.2% in the study of Fieni and al. (2006), whereas it occurs in only two queens in the one of Georgiev and Wherend (2005), which could be related to the earlier administration of aglepristone treatment (day 25 - 26 of pregnancy in Georgiev and Wherend, 2005 versus day 33 in Fieni and al., 2006). However none of the two studies reported any case of uterine infection after aglepristone treatment.
Georgiev and Wherend (2005) reported that 1/23 queen (4.3%) presented itching at the site of injection. In the first study performed by Fieni (Fieni and al., 2006), 8 to 10% of aglepristone injections were painful and approximately 2.5% were followed by increased sensitivity at the injection site or oedema.

3.2.3.2. Hormonal modifications following aglepristone treatment

Few informations can be found in the literature concerning hormonal modifications after pregnancy termination in the queen (Fieni and al., 2006).
No modification in oestrogen, prostaglandin, prolactin or oxytocin plasma concentrations was observed after aglepristone administration. A significant increase of *progesterone and cortisol plasma concentration* was observed respectively 60 hours and 30 hours after treatment with aglepristone.
Termination of pregnancy occurred with high plasmatic progesterone concentration. Foetal expulsion was characterized by an increase in oestrogen, PGFM and oxytocin concentrations whereas prolactin and cortisol levels remained at basal levels.

FELINE PREGNANCY TERMINATION CONCLUSION

Easy to use

Very effective

Valuable *alternative* to ovariohysterectomy

MEDICAL TREATMENT OF CANINE UTERINE DISEASES

INTRODUCTION

Cystic endometrial hyperplasia (CEH), mucometra and pyometra are the three major uterine diseases practitioners will have to deal with.
CEH had been considered for a long time as the first stage of pyometra. However, there are nowadays several pieces of evidence that they should be considered as two separate entities(De Bosschere and al., 2001). Indeed, pyometra is a clinical disease associated with an inflammation of the endometrium, whereas most bitches presenting CEH on ultrasonographic examination will not exhibit any clinical signs.
Mucometra is the accumulation of sterile fluid inside the uterus lumen but present the same ultrasonogaphic pictures than pyometra. However, like CEH, bitches presenting a mucometra will most of time not exhibit any clinical signs and that's why this is most of time diagnosed when routine ovariohysterectomy is performed (Fontbonne, 2007).
Dosage of PGFM can be used to differentiate the three diseases (Hagman and al., 2006). However, this technic is not available in routine practice, so that's why differentiation between the three of them is sometimes hardly done.

Ovariohysterectomy is the treatment of choice. However, *medical treatment* should be considered:
- When dealing with a valuable breeding bitch, as ovariohysterectomy will definitely impair the reproductive function;
- When dealing with bitches that cannot undergo surgery (old bitches with contra-indications for general anaesthesia such as cardiac or renal failure, bitches in very bad general health…).

Luckily, these three diseases share one common point: they are all linked to the *progesterone impregnation* that is present *during diestrus* in the bitches, and therefore, aglepristone can be considered as a valuable medical alternative to treat them.

4.1 PYOMETRA

Accumulation of pus inside the uterine lumen is defined as pyometra and this can become a life threatening disease if not managed on time.

4.1.1 PYOMETRA IN BITCHES

4.1.1.1 Epidemiology

It is most of time diagnosed in adult bitches *around 8.5 years of age* and is more frequent in nulliparous bitches (Niskanen and Thrusfield 1998), but it can happen any time in life, even after the first heats. No breed predisposition was enlightened, but it seemed that crossbred bitches had a tendency to less develop pyometra.
Physiopathology of pyometra is still not clearly understood, but *hormonal impregnation* is clearly a predisposing factors. In one survey, 58% of bitches suffering from pyometra were in diestrus, under progesterone impregnation (Blendinger and al., 1997) and most of time bitches will exhibit *clinical signs from 4 to 12 weeks after their last oestrus*.
As hormonal impregnation of the genital tract seems to play a role in the occurrence of the disease, treatments with estrogens or progestins are a predisposing factor.

4.1.1.2 Clinical expression

Pyometra is a clinical disease associated with inflammation of the endometrium, and can be divided in open or closed cervix pyometra.
When the *cervix is opened*, the main sign observed is a vulvar discharge (from purulent to bloody) and if diagnosis is made early in time, it is often the only clinical sign that is exhibited, with bitches in excellent general condition. The accumulation of pus will then lead to abdominal distension, anorexia, restlessness, renal failure which will be characterized by polyuropolydypsia, vomiting and diarrhea. Hyperthermia can be found but in a very few number of cases.

When the *cervix is closed*, clinical signs are nearly the same (except of course for vulvar discharge) but retention of pus will most of time lead to a more severe expression of the disease and bitches are often presented in very bad condition.

4.1.1.3 Pathophysiology

As we previously said, pathophysiology of pyometra is not well understood, as several factors may act together. However, hormonal impregnation and especially progesterone seems to be one of the major factors in the development of the disease.
Indeed, bitches will be under progesterone impregnation during 9 to 12 weeks after ovulation, and this period is defined as diestrus.
Progesterone influences and stimulates secretion from the endometrial glands and suppresses contractile activity of the myometrium. Moreover, it causes a local immunodeficiency, and also a closing of the cervix. As endometrial secretions accumulate inside the uterus, it creates a good medium for bacteria and can therefore lead to pyometra (Fontbonne, 2007).

4.1.1.4 Medical treatment of canine pyometra

Performing ovariohysterectomy is the treatment of choice and should be proposed first, as it will definitely prevent any recurrence. When medical option is considered, its main goal will be to counterbalance the progesterone impregnation, which is believed to promote the disease, and to remove all the purulent content inside the uterus.
Treatment with PGF2α can be considered as a medical alternative:
- It acts at the uterine level by promoting myometrial contractions, which will lead to an expulsion of the uterine content,
- It also acts at the level of the *corpus luteum* by inducing luteolysis and reducing the blood progesterone level.

The use of prostaglandins is an alternative to surgery, however, side effects are really important (linked to *prostaglandins action on the smooth muscles*: diarrhea, vomiting, panting, restlessness, ptyalism…) and this protocol cannot be used in every situation.

4.1.2. TREATMENT OF PYOMETRA USING AGLEPRISTONE

As progesterone impregnation seems to promote the disease, the use of the progesterone receptor blocker aglepristone was an alternative to explore, as side effects are less important than with prostaglandins F2 alpha.

4.1.2.1 Using aglepristone alone for medical treatment of pyometra

Three different protocols using aglepristone alone can be found in the literature:
- Breitkopf and al. (1997) treated bitches during 16 days with two injections of 5 - 6 mg/kg SC of aglepristone on day 1 (12 hours apart), then one injection of 3 mg/kg SC on days 2, 3 and 4 and in the end one injection of 3 mg/kg SC every 4 days (Breitkopf and al., 1997);

- Blendinger and al. (1997) used two injections of 6 mg/kg of aglepristone on day 1 (12 hours apart) and then administered one injection of 3 mg/kg SC during 3 days (Blendinger and al., 1997);
- Fieni and al. (1998) injected *10 mg/kg of aglepristone once SC on day 1, 2 and 8* and if the uterine lumen was still visible on ultrasonographic examination, another injection was performed on *day 15* (Fieni and al., 1998).

The protocol defined by Fieni and al. (1998) is the one which better fits to pharmacokinetics of aglepristone and is nowadays the one which should be considered.
Breitkopf and al. (1997) reported 85.7% efficiency. Blendinger and al. (1997) performed ovariohysterectomy 6 days after beginning of aglepristone treatment and observed that all the removed uteri were not filled of pus any more. This protocol described by Fieni and al. (1998) was efficient in *77% of the cases after 90 days*, but they reported 61,53% efficiency for open cervix pyometra and 83.33% for closed cervix pyometra. Trach and al. (2003) used the same protocol on 52 bitches and obtained *92.3% of animals that were cured within the first 3 weeks*.

In all studies, the use of aglepristone led to a *cervical opening* 12 to 72 hours after initiating the aglepristone treatment, and vulvar discharge became more important 24 - 36 hours after the first injection. Emptying of the uterine content can be obtained after 6 days (Blendinger and al., 1997).
Improvement of the general condition of the animals is also reported in all the studies 24 - 48 hours after beginning of the treatment and most of time hospitalization is not necessary. Blood parameters (urea, creatinine, WBC) returned to normal in 8 days.

Trash and al. (2003) followed bitches during one year after the medical treatment and observed a *recurrence rate* of pyometra at the following heats in 18.9%, which is similar to results obtained in other studies (Jurka and al., 2010; Fontaine and al., 2009).

4.1.2.2. Using aglepristone in combination with prostaglandins F2α

Fieni and al. (2006) tried to *associate aglepristone and PGF2α* to increase the efficiency of the medical treatment. Two different treatments were administered to bitches suffering from pyometra:
- In the first group, bitches only received aglepristone at the posology of 10 mg/kg on day 1, 2, 8 and 15 if necessary.
- In the second group, they received the same aglepristone injections, but PGF2α analogue cloprostenol was administered at the posology of 1 µg/kg on day 3 to 7.

The mean time to *open the cervix was 25.8 ± 12.2 hours* (4 hours after the 1st injection of aglepristone for the shortest time).

84.4% of bitches were successfully treated in the group combining aglepristone + PGF2α after 90 days, whereas 60% in the aglepristone only group. On Day 15, treatment had been successful in 71.9% of bitches when associating the two drugs, whereas it concerned only 45.3% bitches when aglepristone was used alone.
Gobello and al. (2003) compared two protocols combining aglepristone and cloprostenol (Gobello and al., 2003), with aglepristone at 10 mg/kg on day 1, 2 and 8 (and if necessary on day 15):
- In the first group, cloprostenol was used at the posology of 1 µg/kg from day 3 to day 8.
- In the second group, cloprostenol was used at the same posology on day 3, 5, 8, 10, 12 and 15.

Treatment was efficient in 100% of the treated bitches in both groups, suggesting that the use of cloprostenol from day 3 to 8 was efficient enough to cure pyometra.
Therefore, combining aglepristone and PGF2α significantly increased the success rate of the treatment in a shorter amount of time and this protocol should be used first to medically treat pyometra in bitches. However, due to the side effects reported with the use of prostaglandins, protocols using *aglepristone alone should be used in at risk bitches*, like brachycephalic ones.

4.1.2.3. Using aglepristone in combination with prostaglandins E1

Since PGF2α lead to numerous side effects, the use of PGE1 analogue misoprostol was considered by Romagnoli and al. (2006). Administration of aglepristone was carried out at 10 mg/kg on day 1, 2 and 8 and if complete cure was not achieved, on day 15 and 29. Administration of *misoprostol was carried out at 10 µg/kg twice a day by the owner from day 3 to day 12*. Then if complete cure was not achieved, a final course of misoprostol was administered again from day 29 to day 32. Antibiotherapy was systematically added during 7 days, using amoxicillin/clavulanic acid.

Thus, 13 bitches suffering from open cervix pyometra and 3 from closed cervix pyometra were treated using this protocol. Success (no fluid within the uterus, no vulvar discharge), improvement (improved clinical conditions but still detectable uterine lumen or vulvar discharge) failure and relapse rate were reported (Table 3).

	Success rate (%)	Improvement rate (%)	Failure rate (%)	Relapse rate (%)
Day 15	40	40	20	0
Day 29	62.5	18.8	18.8	0
Day 90	38.5	7.7	23	30.1

Table 3: Success, improvement, failure and relapse rate after treatment of pyometra (Romagnoli and al., 2006).

In the bitches with open cervix pyometra, recovery was characterized by a constant improvement of clinical signs, as well as of uterine conditions and uterine lumen diameters. In the three closed cervix pyometra bitches a worsening of clinical signs was first observed, coincident with cervical opening (12 - 48 hours after the first aglepristone injection) and onset of vulvar discharge; however general conditions rapidly improved thereafter and by day 29, one had recovered completely while the 2 others showed a clear improvement; however at day 90 signs of pyometra reccured in the three of them.

Administration of aglepristone and misoprostol induced return to normal conditions or a *significant clinical and uterine improvement in more than 75% of the cases by day 29*. Treatment tolerance was good. Owner compliance was also good due to the ease of misoprostol administration as well as the scarcity of its side effects, mostly only on the first day of treatment (vomiting in 25% of cases; diarrhea in 31% of cases). A combined treatment of aglepristone can therefore be used in case of a closed or open cervix pyometra both as a first choice therapy for valuable breeding bitches as well as to improve general clinical conditions prior to ovariohysterectomy in otherwise poor surgical candidates.

4.1.2.4. Recurrence of the disease

If ovariohysterectomy definitely prevents recurrence of the disease, bitches treated medically can still develop pyometra as soon as progesterone impregnation is back, which means at the following diestrus period.
Recurrence of the disease was observed in 18.9 to 27% of the cases (Gobello and al., 2003; Trasch and al., 2003; Jurka and al., 2010; Fontaine and al., 2009).
Pregnancy is considered to be the best way to prevent recurrence, that's why bitches that were medically treated should be bred on the following heat.

4.1.2.5. Fertility results after medical treatment of pyometra

As it is mentioned in studies focusing on pregnancy termination, aglepristone treatment may shorten the interoestrus interval by 1 to 3 months (Trasch and al., 2003; Jurka and al., 2010; Fontaine and al., 2009).
In the study of Trasch and al. (2003), 7 bitches were bred and 6 of them delivered normal litters. Jurka and al. (2010) reported a 57.1% pregnancy rate, with litters from 1 to 12 puppies, and observed that bitches above 5 years of age did not conceive.
Fontaine and al. (2009) reported *80.1% pregnancy after medical treatment of pyometra using aglepristone* with mean litter size of 4.5 ± 3.6 puppies (from 1 to 11). On the contrary to Jurka and al. (2010), no effect of the age was observed, and bitches above 5 years conceived. These differences may be explained by the fact that, in this study, all bitches went through a timing of ovulation to determine the best breeding period. There was a tendency for giant bitches to have smaller litters, but as very few cases were considered, these results remain to be ascertained.

This treatment was therefore proved to be efficient in maintaining fertility capacities of the bitches.

4.1.2.6 Suggested protocol for medical treatment of pyometra in the bitch

- Administer aglepristone at the *posology of 10 mg/kg SC on day 1, 2 and 8*. Further injections can be performed on day 15 and 21 if some liquid is still present in the uterine lumen.
- Administer *prostaglandins F2α* (cloprostenol, 1 µg/kg SC or dinoprost, 150 - 200 µg/kg SC) from day 3 to day 8 or *prostaglandin E* (misoprostol 10 µg/kg twice a day PO) from day 3 to day 12; to decrease side effects related to prostaglandins injection, administration of butylscopolamine or association of prifinial-atropine-metopimazine should be administered 15 minutes before; the animals should be kept under veterinary control during 20 - 30 minutes after prostaglandins administration.
- *Antibiotherapy* should be instaured during the whole length of the treatment (amoxicillin-clavulanic acid or cefalexine should be used first); performing an antibioresistance profile is not mandatory.
- If the general health is altered at the time of diagnosis, the bitch should be *hospitalized* to perform *fluidotherapy* and adjunctive cares.
- *Progesterone rate* should be performed before initiating the treatment. Indeed, aglepristone is a progesterone receptor blocker and it will mainly be efficient if progesterone impregnation is clearly demonstrated in the bitch suffering from the disease. However, in everyday practice, we are aware that this is not always performed.
- *Ultrasonographic examinations* should be performed on day 8, 15 and 21 to assess the effect of the medical treatment. Presence of fluid inside the uterine lumen should motivate administration of aglepristone ± prostaglandins F2α.
- During the whole treatment, the bitch should wear an *Elizabethan collar* to avoid licking of the vulvar discharge, as this can lead to disseminated intravascular coagulation, especially during the first week when discharge will be increased.
- It is necessary to *strictly monitor health* during the entire treatment time, especially during the first week; if no purulent vulvar discharge is noticed by the owner, a control ultrasonographic examination should be performed and if presence of fluid is still observed, medical treatment has to be stopped as it can lead to uterine rupture. Ovariohysterectomy might then be considered.
- As around *20% of bitches will exhibit recurrence* of the disease during the following diestrus, if possible, breeding should be encouraged at the next heats, as pregnancy most of time prevents development of pyometra. A careful breeding management (timing of ovulation to determine the best breeding period) is mandatory to increase success rate. After breeding, careful monitoring of the bitch should be proposed to the owner (ultrasonographic examinations once a week) to detect early signs of pyometra and considered how to manage it if present.

4.2 CYSTIC ENDOMETRIAL HYPERPLASIA (CEH)

CEH was considered for a long time as the first stage of pyometra, but there are now several pieces of evidence that the two entities should be separated (De Bosschere and al., 2001).
CEH is a *non clinical disease*, as bitches will not exhibit any clinical sign. It is commonly found in old bitches during routine ultrasonographic examination, and is characterized by presence of cysts in the endometrium associated with a slight uterine enlargement.

4.2.1. CLINICAL DATA

This disease is related to *progesterone impregnation* and was often called metritis in an improper way, as no inflammatory process is involved in its pathophysiology (Hagman and al., 2006).
Medical treatment using aglepristone was considered (Fieni, 2006), using aglepristone injections on day 1, 2, 8 and if necessary on day 15 and 21.
15 bitches that were included were successfully treated. Fontaine and al. (2009) reported pregnancies on two bitches suffering from CEH after medical treatment with litters of 5 and 7 puppies.

4.2.2. SUGGESTED PROTOCOL FOR TREATMENT OF CEH

- ➜ *Progesterone rate* should be first determined, to ascertain the utility of aglepristone treatment. However, we are aware that this is not always done in routine practice.
- ➜ Aglepristone should be administered at the *posology of 10 mg/kg SC on day 1, 2, 8*. If presence of cysts is still assessed on ultrasonographic examination, it should also be performed on day 15 and 21.
- ➜ *Ultrasonographic examination* should be performed once a week to assess improvement, and aglepristone should be administered till no more cysts are visible in the endometrium.
- ➜ No adjunctive antibiotherapy is needed, as CEH is a non inflammatory process.

4.3. MUCOMETRA

Mucometra is accumulation of sterile fluid inside the uterine horns, usually not associated with clinical signs. However, as uterine enlargement can be important, it can hardly be differentiated with closed cervix pyometra on ultrasonographic examination.
Even if mucometra is linked to progesterone impregnation, there are no reports of medical treatment using aglepristone in the literature. Moreover, veterinarians have to be aware that mucometra may not respond to aglepristone treatment (Fontaine and al., 2009) on the contrary to pyometra, where more than 90% efficiency is usually recorded.

Therefore, *mucometra should be considered when veterinarians will encounter a failure in aglepristone treatment with a bitch presenting a uterine enlargement*.
If ovariohysterectomy has to be avoided, other options, like using transcervical catheterization of the uterus to empty it from its content (Verstegen and al., 2008), might be considered.

MEDICAL TREATMENT OF FELINE UTERINE DISEASES

Uterine diseases are not *very common in queens*, maybe because progesterone impregnation is less important in this species. Indeed, *corpus luteum* will develop only after mating, which is induced by ovulation in most of cases.
However, cystic endometrial hyperplasia (CEH), mucometra and pyometra may be encountered and are described.
As in bitches, hormonal influences, mainly related to progesterone, result in environmental and histological changes at the uterine level, leading to a predisposition to bacterial growth.
Ovariohysterectomy is also the treatment of choice, but medical alternative may be considered when dealing with elder animals that cannot undergo surgery or valuable breeding queens, and aglepristone appears as a treatment of choice.

5.1. CLINICAL RESULTS

Aglepristone was successfully used in queens to induce mid pregnancy termination (Fieni and al., 2006) or to treat mammary fibroadenomatosis (Görlinger and al., 2002), but there is only one report concerning its use for pyometras (Nak and al., 2009).
Ten cats, 2 - 13 years of age, suffering from pyometra were treated with aglepristone.
Administration of 10 mg/kg SC of *aglepristone was performed on day 1, 2 and 7*. Another injection was performed on day 14 if presence of fluid was still noticed in the uterus at the ultrasonographic examination. In addition, antibiotherapy (thrimetoprim/sulphadoxine) was also administered for 7 days.

Nine cats out of 10 responded well to treatment. No recurrence was observed in a *follow up period of two years and no side effects were observed*.
These data suggest that aglepristone treatment is a promising approach for medical treatment of pyometra in cats. The 90% success rate was obtained with aglepristone alone and using a posology of 10 mg/kg, which is different to the one recommended by *the pharmacokinetics data (15 mg/kg)*.

Unfortunately a lot of clinical data are lacking in the queen, like the benefit of combining prostaglandin F2α and aglepristone, recurrence rate and fertility results after medical treatment, and more studies have to be carried out on the topic to enlighten those fields.
Nothing is also available in the literature concerning treatment of CEH and mucometra with aglepristone in the queen.

5.2. SUGGESTED PROTOCOLS FOR MEDICAL TREATMENT OF FELINE UTERINE DISEASES WITH AGLEPRISTONE

- Aglepristone should be administered on *day 1, 2 and 7* at the dosage of *15 mg/kg SC*. Administration on day 15 and 21 may be considered if presence of fluid is still visible at the ultrasonographic examination.
- *Ultrasonographic examination* should be performed on day 8, 15 and 21 after beginning of the treatment. If efficiency is doubtful (no purulent discharge observed by the owner, no modification of the uterine enlargement on ultrasound picture), treatment should be stopped and ovariohysterectomy might be considered.
- Antibiotherapy might be instaured when pyometra is suspected.
- If purulent vulvar discharge is present, the queen should wear an *Elizabethan collar* to avoid ingestion of the purulent content.
- As in the bitch, recurrence may happen and it is also recommended to breed the animal to prevent this risk. Therefore it appears necessary to carefully monitor the queen after breeding, and as in the bitch, an ultrasonographic examination is recommended once a week.

CANINE PARTURITION

6.1 PHYSIOLOGY OF PARTURITION

6.1.1. ROLE OF PROGESTERONE

Copora lutea are the sole source of progesterone in the bitch and are mandatory to maintain pregnancy. Indeed, *progesterone is responsible of the quiescent state of the myometrium and closing of the cervix*. Changes in the myometrial contractions that will lead to parturition are correlated to the drop in progesterone levels (Figure 16), that occurs 24 hours before parturition (Van Der Weyden and al., 1989).

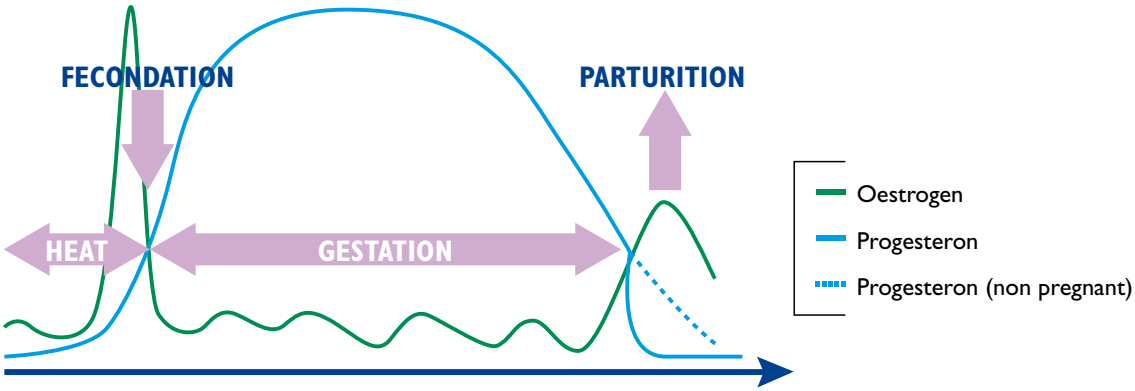


Figure 16: Progesterone and oestrogen concentrations during gestation.

6.1.2. ROLE OF OESTROGENS

Œstrogen secretion promotes *contractility of the myometrium* and therefore, myometrial function will vary depending on their ratio with progesterone (Stabenfeldt and Davidson, 2002). Inversion of this ratio will occur as progesterone drops at the end of pregnancy.
Œstrogen secretion will also promote *secretion of PGF2α* by the endometrium. These prostaglandins will enhance myometrial contractions and cervical dilatation. Moreover, due to their luteolytic effect, they will also lead to a decrease of the progesterone rate, which will make the endometrium more sensitive to prostaglandin action.
Œstrogens will also increase *the number of oxytocin receptors* at the endometrial level.

6.1.3 ROLE OF OXYCTOCIN

Oxytocin *increases contractions* from the myometrium and will be secreted at the pituitary level when fetuses will go through the cervical canal. This neurological phenomenon is known as "Ferguson's reflex" and actively participates to the parturition process.

Therefore, parturition is a multifactorial process, where several hormones play a critical role.
If the triggering factor is of foetal origin, the *drop in progesterone level is mandatory* for inversion of the œstrogen-progesterone ratio (it is considered that progesterone level has to be below 2 ng/mL for parturition to begin). Afterwards, œstrogen, oxytocin and prostaglandins will act together to permit parturition.

6.2. INDUCTION OF PARTURITION IN THE BITCH

Induction of parturition may be indicated for medical reasons, in maternal or fetal interest:
- *Increased pregnancy length* with no sign of ongoing parturition (for example bitches that did not whelp after 65 days post-ovulation)
- *High risk of uterine inertia*: primiparous bitches above 4 years of age, some predisposed breeds (poodles, Scottish terriers), small sized litters especially in large and giant breeds ("single puppy syndrome") (Fontaine and al., 2008).

Moreover, two bitches out of three will deliver in the middle of the night. Thus, for evident convenient reasons, being able to plan in an accurate way the time of parturition during the day could make management of whelping bitches more efficient.

This procedure is more likely to be successful if physiological mechanisms are replicated.

6.2.1. MEDICAL APPROACH TO INDUCE PARTURITION IN THE BITCH

In order to fit to *the physiological mechanisms*, a reliable protocol to induce parturition in the bitch has to enable hormonal events related to the drop of progesterone level. Ideally, the chosen drug should induce whelping with a high efficiency and within a predictable short time frame after treatment. In addition, treatment should be safe for the bitch and her puppies and should induce a normal parturition without side effects.

As we previously saw in the chapter concerning abortion, several ways could be tried:

- Obtaining luteolysis:
- By the use of *antiprolactins*: the use of the dopamine agonist cabergoline or bromocryptine will induce luteolysis by suppressing the release of prolactin. However, dopamine agonists are not useful for the induction of whelping since an effect may be expected only after *several days*. In addition, the time between the start of dopamine agonist treatment and the onset of parturition is quite *unpredictable*. Furthermore, treatment with prolactin secretion inhibitors would reduce or even *abolish lactation* after parturition. Therefore antiprolactins cannot be considered for medical induction of parturition.
- By the use of *prostaglandins F2α* and its synthetic analogues (cloprostenol for example): these compounds will also induce regression of the *corpora lutea* resulting in the termination of pregnancy. Just as dopamine agonists, prostaglandins must be administered for *several days* before luteolysis takes place in the bitch. In addition, treatment with prostaglandins is often accompanied by *side effects* such as tachypnea, salivation, vomiting and diarrhea and there may be an increase risk of an abnormal parturition process (Eilts, 2002).

- **Mimicking the drop of progesterone level by using progesterone receptors blockers:** mifepristone and aglepristone are competitive antagonists of the progesterone receptors. Because of their antiprogesterone effects, these drugs have been widely investigated for their use as abortifacient agents and may in addition be useful for the induction of whelping as well.

6.2.1.1. Use of antiprogestins alone

Mifepristone had been used to induce parturition in woman suffering for uterine inertia and pre-eclampsia and was efficient in 60% of cases without human assistance (Frydman and al., 1991). In the bitch, it was noticed that the use of mifepristone did not lead to prostaglandin release from the endometrium, as these values tended to stay at basal levels, which could explain a lack of contractions (Nohr and al., 1993).

Baan and al. (2008) administered aglepristone (10 mg/kg SC) twice 9 hours apart to induce parturition in beagle bitches on day 58 of pregnancy and compared an induced group to bitches whelping without any medical assistance. Adjunction of oxytocin was performed when interval between two pups was longer than 1 hour. Induction of parturition was successful in all treated bitches and delay of expulsion and puppies viability were not statistically different. However, 5 bitches out of 6 needed at least two oxytocin injections, which tends to confirm that *aglepristone alone also leads to a lack of contractions*.

Therefore, mifepristone and aglepristone were efficient for induction of parturition but not in expulsion of the fetuses. *Their association with uterotonic compounds could be interesting to obtain a quicker induction and more regular contractions for a normal whelping process*.

6.2.1.2. Use of combination of antiprogestin and uterotonic compound

There are no reports concerning the use of mifepristone and an uterotonic compound to induce parturition in the bitch, all studies that were realized concerned aglepristone.

6.2.1.2.1. Combining aglepristone and prostaglandins F2α

Two studies stated about the association between aglepristone and prostaglandins F2α (Hoffmann and al., 1999; Fieni and al., 2001).
On the 58th day of pregnancy, Hoffmann and al. (1999) administered aglepristone (10 mg/kg SC) to one pregnant bitch; another injection was realized 24 hours later. 12 hours after the first administration of aglepristone, PGF2α (dinoprost, 160 µg/kg SC) was added; another injection was realized 12 hours later. The first pup was expulsed 4 hours after the last prostaglandin administration, quickly followed by two other puppies. After a 2 hours break, the bitch received 0.5 IU of oxytocin and delivered the two last puppies. The five of them were healthy and no side effects were observed on the mother.

Fieni and al. (2001) administered aglepristone (15 mg/kg SC) on the 58th day of pregnancy to beagle bitches. 24 hours later, the treated bitches received 0.08 mg/kg aflaprostol (a prostaglandin F2α analogue) every two hours and this was realized until the expulsion of the last pup. Parturition occurred in all bitches between 27.3 and 37.8 hours after administration of aglepristone (95% of bitches delivered between 30 and 34 hours after aglepristone administration) and expulsion length between the first and the last pup was 9.1 ± 2 hours. 68% of the puppies survived 48 hours after parturition.

6.2.1.2.2. Combining aglepristone and oxytocin

Fieni and al. (2001) also associated aglepristone with oxytocin instead of prostaglandins F2α. *0.15 IU of oxytocin was administered every 2 hours 24 hours after aglepristone administration (15 mg/kg SC)*. Time to the onset of parturition was not significantly different than in bitches treated with prostaglandins. However, expulsion length was significantly shorter (4.5 ± 1.8 hours versus 9.1 ± 2 hours with PGF2α) and survival rate 48 hours after birth was also significantly higher (86.21% versus 68% in bitches treated with PGF2α). In conclusion, *the protocol combining aglepristone and oxytocin was proved to be safer and with a significantly shorter expulsion time than the association with PGF2α*.
The same protocol was applied to a larger number of bitches (Fieni and Gogny, 2008). 22 mature beagle dogs were used. Parturition was obtained in all bitches at an average of 29.7 ± 5.6 hours after aglepristone administration, the shortest interval being 15 hours and the longest 40.5 hours. Average expulsion time at parturition was 5.9 ± 1.9 hours, which was equivalent to an average of 1.1 ± 0.4 hours per pup; 121 pups were born. The frequency of live pups was 89.1% 48 hours after birth and 86.5% 7 weeks after birth. On average, 0.72 live pups at birth subsequently died per litter. These results confirmed the ones obtained in the previous study, showing the *efficiency and the reliability of this protocol*.

However, all these studies were performed in beagle bitches, and one can wonder what would happen in other kind of bitches. Fontbonne and al. (2008) applied the protocol combining aglepristone and oxytocin to various sized bitches belonging to *different breeds* in order to confirm if this medical induction of parturition remained effective in other canine breeds. 13 pluriparous bitches were included, with small, large and giant sized bitches, some were treated to induce parturition and the others did not receive any kind of medication to serve as controls.
The first pup was born 25.9 ± 3.29 hours after aglepristone administration (range from 21 to 30 hours). 2/7 bitches (belonging to the small size group) with respectively 5/8 and ¼ pups, were born before the first administration of oxytocin. In the induced group, 6/7 bitches required no human assistance during parturition. Parturition length was 9.6 ± 5.4 hours, ranging from 3.7 hours to 17.5 hours. The mean duration of parturition was shorter in small bitches (3.8 hours) than in large (11.2 hours) or giant (14 hours). The mean interval between two successive fœtal expulsions was 115.6 ± 82.8 min (versus 68.8 ± 24.5 min in the control group). After 48 hours, 6.1 ± 3.4 pups were alive in the induced group (versus 7 ± 2.4 in the control group). The mean weight at parturition did not differ significantly between the two groups (355 ± 174 g in the induced group versus 363.3 ± 176 g in the control group). All pups gained weight during the first 48 hours except four small size pups from the induced group which looked premature at the time of birth and died between 19 to 29 hours post delivery. This study shows that a *protocol combining aglepristone + oxytocin successfully induces parturition in bitches, whatever size and breed*.

The mean duration of induced parturition was however longer that what had been found by Fieni and al. (2001) using the same protocol in Beagle bitches and this was especially true in large and giant breeds. The mean interval between two successive births of pups was longer in the induced group. Therefore, the authors concluded that *this protocol cannot fully predict the duration of the entire parturition process*, as it seemed to be the case in beagles. These observations might suggest that when parturition is induced in small bitches, the birth of the first pup may occur sooner than expected, before the first administration of the uterotonic compound. The death of the four apparently premature Yorkshire terriers pups raises the potential risk of inducing parturition too prematurely. This should therefore be used cautiously and emphasizes *the need for veterinarians to have detected the time of ovulation as precisely as possible before*.

6.2.2. HORMONAL MODIFICATIONS AFTER MEDICAL INDUCTION OF PARTURITION USING ANTIPROGESTINS

6.2.2.1. Progesterone

Increase in progesterone plasmatic level is reported in the 8 hours following treatment when aglepristone is used to induce parturition. This increase can be explained by the fact that aglepristone binds to progesterone receptors with a greater affinity than the natural steroid hormone. Moreover, as aglepristone mimicks a decrease in the progesterone rate and therefore no more negative feedback at the hypothalamus level, this may stimulate secretion of LH and FSH at the pituitary, leading to an increase secretion of progesterone. Parturition will begin with *progesterone levels higher than 2.7 ng/ml*, which is similar to what is found when used for pregnancy termination (Baan and al., 2008).

6.2.2.2. Prostaglandins

4 hours after aglepristone administration, *an increase in PGFM level* is reported; however, PGFM values are lower than compared to a natural parturition (Fieni and al., 2001). Baan and al. (2008) reported that PGFM concentrations increased before parturition in both bitches delivering without any medical treatment and induced ones, but levels were lower in the induced bitches. PGFM levels reached a maximum in both groups during parturition and quickly decreased in the spontaneously whelping group after parturition, but remained elevated in the induced group.

Aglepristone alone is therefore *not able to mimick the exact hormonal events that occurred during normal parturition process* (Baan and al., 2004). This is why an *uterotonic agent should be added to enhance myometrial contractions*, in order to increase efficiency of the protocol.

6.2.2.3. Cortisol

Cortisol concentrations reached similar maximum levels during the last 30 hours before the onset of expulsion when bitches are induced with aglepristone or not. However, during the 3 days post-partum, cortisol concentrations were higher in the induced group. This may be due to the partial blocking of pituitary glucocorticoid receptors by aglepristone. Elevated postpartum cortisol concentrations in the induced group may also have been caused by a sustained postpartum PGF2α production as reflected in the elevated PGFM concentrations. However, there was *no clinical evidence for an elevated stress response in the induced bitches*, as post-partum behavior, the number of post natal losses and the weight increase of the puppies were similar to those spontaneously whelping dogs.

6.2.2.4. Prolactin

20 hours following aglepristone injection, *a rise in prolactine concentration* is observed, resulting of the arrest of a negative feedback at the hypothalamic level linked to progesterone. This peak of prolactin concentration is similar to what happens in natural whelpings and can explain that *lactation may occur sooner* in those treated bitches.

Therefore, these data illustrate the hormonal changes after induced parturition in the bitch and reveal that the use of aglepristone is associated with still incomplete luteolysis, an altered PGFM profile, an elevated post partum cortisol concentrations as compared with spontaneously whelping dogs (Baan and al., 2008).

6.2.3. RECOMMENDATIONS FOR INDUCTION OF PARTURITION USING AGLEPRISTONE

6.2.3.1. Protocol

- According to data found in the literature, the protocol combining *aglepristone (one injection of 15 mg/kg SC) and oxytocin (0.15 IU/kg every two hours, 24 hours after aglepristone administration)* was proved to be more efficient and safer than the others, and is the one that is currently recommended when such indication should be used.
- One critical point is to define the optimal time of aglepristone injection and to do so, a *precise timing of ovulation using progesterone quantitative assays* should have been performed during the heat period. As the limit of prematurity is around 58 days (Fontaine and al., 2008) and pregnancy length in the bitch is 63 ± 1 day (Tsutsui and al., 2006) post ovulation, *parturition should be induced between 58 and 60 days post-ovulation*.
- The *number of pups should be precisely known* before applying the medical protocol, so an *X-Ray* should be performed before.
- *A progesterone rate* should be performed prior applying the medical protocol. If the bitch is below 2 ng/ml, parturition is imminent and there is no need for a medical treatment.
- As *careful management of parturition* is needed during medical induction of whelping, the all procedure should be realized in a veterinary clinic and the bitch should be hospitalized several days before to lower the stress level that can influence parturition process.
- In beagle bitches and in large and giant ones, parturition usually began 24 hours after administration of aglepristone, after administration of the uterotonic agent. However, practitioners have to be aware that in *small sized bitches the process may begin before* and without any injection of oxytocin. These bitches should therefore be carefully monitored.

6.2.3.2. When to use ?

Medical induction of parturition using aglepristone should be considered:
- To reduce morbidity and foetomaternal mortality
- To prevent some well defined pathologic cases of parturition, among which:
  - Exceeded term/prolonged gestation (> 63 days post ovulation, > 67 days post mating)
  - To prevent some expulsive defects (uterine inertia)

Although good results were obtained during clinical trials this protocol may not be use as a convenience programmation and the practitioner might consider its use in regards to ethical purposes.

6.3. PLANNING ELECTIVE CAESAREAN SECTION WITH AGLEPRISTONE

Nowadays, owners expect to have high rates of pregnancy and survival of offspring. Several studies investigated the overall incidence rate of dystocia (5 to 16% of pregnancies) and the risk factors leading to dystocia (breed, age, number of puppies …) (Bergström and al., 2006; Fontaine and al., 2008). *60 to 70% of dystocia ended with caesarean sections* (Bergström and al., 2006).

Moreover, some studies evaluated the perioperative risk factors affecting neonatal survival after caesarean sections and confirmed what had been described in human obstetrics: *a caesarean section (CS) performed in emergency does result in higher risk* to the dam and the foetuses compared to a planed CS (Moon and al., 2000). The likelihood of having all puppies remaining alive at birth after an emergency surgery is only one third as when the surgery is planned. (Moon and al., 2000).

Traditionally, using progesterone assays and rectal temperature follow-up, in combination with a carefully timed and documented breeding management, it is possible to plan a caesarean section. But these methods may be costly and time consuming for owners and veterinarians: numerous progesterone assays during the last days to define the drop under 2 ng/ml; they don't allow to schedule a caesarean section at a given time many days in advance; and sometimes, a caesarean section has to be performed at the very last minute in the evening or in the night.

Taking into account that protocols in order to induce parturition with aglepristone around 60 days after ovulation (before the drop of progesterone blood level below 2 ng/ml) were described in the literature (Fieni and al., 2001; Baan and al., 2005), Levy and al. (2009) tried to schedule caesarean section, before the natural initiation of parturition.

6.3.1. PROTOCOL DESCRIBED FOR PLANNING ELECTIVE CAESAREAN SECTION USING AGLEPRISTONE

Timing of ovulation was performed during the oestrus period in order to determine the ovulation day. Then, based on the protocol described by Fieni and al. for induction of parturition (2001), *aglepristone was administered at the posology of 15 mg/kg on day 60 after ovulation and caesarean section was performed around 20 hours after*.
37 bitches were included and none of them showed signs of parturition before the time surgery was planned. *Progesterone remained above 2 ng/ml* (mean = 5.25, SD = 1.28, min = 2.4, max = 7.2) in all bitches and no unexpected side effect related to the aglepristone treatment was observed in any of them. No suffering foetuses were observed by abdominal ultrasonographic examination before caesarean section.

The mean litter size was 5 (SD = 2.6, min = 1, max = 10). 5 out of 188 puppies died in the first two weeks (2.6%) belonging to three different bitches: English bulldog (1 out of 4 puppies), French bulldog (1 out of 4 puppies), and beagle (3 out of 7 puppies): one French bulldog pup was found partially eaten by its mother in the first hours of life (a discrete "water puppy syndrome" was present in this pup at the time of CS: slight oedema of the hindlimbs). One English bulldog puppy died at 5 days from important untreated cleft palate. Three beagle pups died in the first 15 days of life (D9 – D10 – D11). After necropsy, the death of beagle's neonates was most probably due to neonatal septicaemia (weight decrease, failure to suckle, severe diarrhea) but could not be confirmed by bacteriological analysis.
No post-operative clinical complication was reported in any of the bitches. 33 bitches *excreted a lot of milk at the end of the CS* to nurse their puppies and 4 bitches started to milk the next day (16 to 24 hours post CS) only: 3 Beagles bitches and one English bulldog. Pups were bottle fed by a commercial milk replacer every 2 hours while awaiting the milk production by the dam. No neonate showed any clinical signs of prematurity and they were all vigorous.

The percentage of death in the first 15 days (2.6%) was low compared to data found in case of CS performed in emergency (13 to 20%) or natural delivery (25%) (Moon and al., 2000; Fontaine and al., 2007). Furthermore, the neonatal deaths found did not seem to be strictly related to the CS and/or the protocol: one pup died from a "water puppy syndrome", one pup was killed in the first 48 hours, one pup died from cleft palate, and the three others, that showed a previous normal growth and behaviour, died (fading puppy syndrom) between 9 and 11 days. The neonates who died belonged to a bitch that was milking at the time of C-section.

6.3.2. WHY USING AGLEPRISTONE ?

Most authors recommend waiting until the drop of progesterone (< 2 ng/ml) to perform CS (Smith, 2007). However, the triggering mechanism for parturition remains unclear in the bitch and no one has confirmed that the final drop of progesterone to basal value was a necessary signal for the terminal foetal maturation (surfactant release…) before parturition. Moreover, recent publications tend to prove that surfactant had already been released on 58th day of pregnancy, meaning that the *progesterone drop do not play any role in final foetal maturation* (Kutzler and Volkmann, 2008). Therefore, one may wonder if aglepristone use is mandatory in this indication, and if adequate timing of ovulation will not be enough to plan the time of surgery.
However, hormonal changes that occur after the use of aglepristone may play an important role. As prolactin secretion is increased, it may play an *essential role in stimulation of lactation*. Indeed, in this study, 33/37 bitches (89.1%) produced a lot of milk at the time of caesarean section, without a final drop of progesterone and/or vaginal delivery. Aglepristone administration could be suggested in order to increase milk production, but one should keep in mind that, in our study, four bitches remained without any milk secretion (agalactia) during the first 24 hours post caesarean section.

6.3.3 RECOMMENDATIONS FOR PLANNING CAESAREAN SECTION WITH AGLEPRISTONE

- *Adequate timing of ovulation* has to be performed in order to determine the day of surgery in the most adequate way. Practitioners have to be aware that if done too early, puppies might not be mature enough and will quickly die, whereas if performed too late, parturition might begin before the planned time and all the advantages given by the use of this procedure will be lost.
- Aglepristone has to be administered *60 days post-ovulation* at the posology of *15 mg/kg* and caesarean section should be performed *around 20 hours after*.
- Excellent results (especially a very low rate of neonatal mortality) was obtained in the study of Levy and al. (2009). However, veterinarians have to be aware that these results are not only linked to the medical approach, but also to the *efficiency of the anaesthetic, surgical and resuscitation procedures* that were subsequently performed. As caesarean section is planned, it becomes easier to prepare an entire technical staff to master all these points that are critical in the success of the procedure.

FELINE FIBROADENOMATOSIS

Feline fibroadenomatosis (also known in the literature as fibroadenomatous hyperplasia, feline hypertrophy of teats or fibroglandular hypertrophy of teats) is characterized by a rapid and abnormal growth of one or more mammary glands. This affection is only reported in the cat (Hayden and al., 1981), mainly intact females are affected but neutered queens and even male cats may suffer from this disease. This *non neoplasic process* is linked to stromal proliferation of the mammary gland epithelium.

7.1. FELINE FIBROADENOMATOSIS

7.1.1. CLINICAL EXPRESSION

7.1.1.1. Clinical signs

The animal may be presented with enlarged and inflamed mammary glands. In 87% cases, only the inguinal mammary glands are affected. These lesions will lead to local ulceration and become painful. *General condition* is usually not altered but tachycardia, lethargy, and sometimes anorexia may occur. However, *consequences of local complications* (especially ulcerations) may affect the animal's health.

7.1.1.2 Affected animals

75% of queens presenting fibroadenomatosis are young queens that had ovulated and are sometimes pregnant (Hayden and al., 1981). In rare cases, this can occur in neutered queens or even in males after exogenous administration of progestins.

7.1.2 PATHOPHYSIOLOGY OF FELINE FIBROADENOMATOSIS

There are several pieces of evidence that *progesterone impregnation* is responsible of the onset of feline fibroadenomatosis.

First of all, progesterone receptors were found in all affected mammary glands and were located in the non luminal suprabasal epithelial cells and in the stromal cells (Martin de las Mulas and al., 2000), which role is to increase the size of the gland. *Progesterone may therefore stimulate mammary growth* at their level. Growth Hormone receptors can also be found at the same level, and as progesterone may enhance local production of this hormone, this will also lead to mammary hypertrophy (Ordas and al., 2004).

Moreover, administration of progestins (medroxyprogesterone acetate, proligestone) on elder or neutered queens and even in males can lead to fibroadenomatosis. Lobular mammary hyperplasia was also obtained in 18% queens treated with megestrol acetate versus 3% in untreated ones (Hayden and al., 1989).

Therefore, treatment of this disease could only be successful by *removing the progesterone impregnation at the mammary gland level*.

7.1.3. TREATMENT OF FELINE FIBROADENOMATOSIS

Ovariectomy is a possible treatment in affected females and after this procedure, the mammary tissue will decrease in size and recovery will be completed 3 to 4 weeks after. However, failure is possible and this could not be proposed in breeding queens or when the owners refuse surgical neutering.

7.2. TREATMENT OF FELINE FIBROADENOMATOUS USING AGLEPRISTONE

7.2.1. CLINICAL RESULTS

Antiprogestins are of great interest to suppress progesterone impregnation.

Several studies reported the efficiency of Aglepristone. Wehrend and al. (2001) treated 6 queens with administration of 10 mg/kg aglepristone SC during 4 consecutive days. 5 days after onset of the treatment, *decrease in size of the mammary glands* was observed and recovery was obtained in 3 to 4 weeks, as it would have been observed with ovariectomy (Werhend and al., 2003). Another protocol was proposed by Görlinger and al. (2002). Queens received 10 mg/kg aglepristone twice 24 hours apart once a week or 20 mg/kg SC once a week. Aglepristone was administered until complete recovery was obtained. *95% of queens were successfully treated* and no *side effects were reported*, except local irritation at the injection place in two cats (Görlinger and al., 2002), which is commonly reported in all species where aglepristone was used at this dosage. In the same study, a male cat that received progestins was successfully treated with the same protocol. However, recurrence was reported 13 days after the end of treatment, suggesting that *in presence of progestins*, treatment with *aglepristone should be maintained* until these compounds do not act any more, their half life being longer than natural progesterone. Therefore some authors recommend a *5 week treatment at least when progestins were given* (Jurka and Max, 2009).

Jurka and al. (2009) reported *fertility results* and after treating fibroadenomatosis with the protocol previously reported, 6 queens were bred. 4 queens went pregnant and delivered normally, no signs of recurrence were noted. Aglepristone had no effect on fertility parameters, as it is described in the bitch.

In this last study, the disease relapsed in the two queens that were not pregnant. Reports on *recurrence* of fibroadenomatosis are very rare and some thinks it is quite uncommon, but authors recommend surgical neutering in case of relapses.

7.2.2. RECOMMENDATIONS FOR MEDICAL TREATMENT OF FELINE FIBROADENOMATOSIS USING AGLEPRISTONE

- As development of the disease is related to progesterone impregnation and most of queens will ovulate after mating, ultrasonographic examination is recommended to check pregnancy before initiating the treatment. Indeed, aglepristone may lead to abortion that could affect the owner if not prepared.
- The protocol proposed by Gorlinger and al. (2002) gave excellent results and is the one that is currently recommended. However, the dose that was used (10 mg/kg SC) was different from the one suggested by the *pharmacokinetics data on aglepristone (15 mg/kg)*.
- Aglepristone can therefore be administered at the dosage of *10 mg/kg twice 24 hours apart once a week until complete recovery is obtained*. In case the queen is pregnant, 15 mg/kg can be used to achieve satisfactory pregnancy termination.
- In case the disease is linked to *progestins administration*, aglepristone has to be administered every week until progestin is active at the mammary gland level, as recurrence may happen as soon as aglepristone action ended.
- If medical option is not successful, *ovariectomy* should be performed ± *mastectomy* if ulcerative lesions occurred. If ovariectomy does not solve the problem, mastectomy of the affected glands has to be performed.

Aglepristone is therefore a good alternative to ovariectomy in the treatment of fibroadenomatosis, being efficient in around 4 weeks and with no side effects reported. This is the treatment of choice in breeding queens.

8 OTHER INDICATIONS REPORTED IN THE LITERATURE

8.1. SHORTENING OF INTEROESTRUS INTERVAL

Most of studies reporting the use of aglepristone showed that interoestrus interval was shortened by 1 to 3 months (see Canine termination of pregnancy chapter) and this is believed to be related to an effect on the hypothalamo-pituitary axis.
Galac and al. (2004) focused specifically on this topic and administered aglepristone once daily on two consecutive days in a dose of 10 mg/kg every week until the end of the luteal phase, beginning 12 ± 1 days after ovulation.
The differences they reported between in mean plasma concentration of progesterone and prolactin before, during and after treatment were not significant. Also, the duration of the luteal phase in the treated bitches (72 ± 6 days) did not differ significantly from the untreated ones (74 ± 4 days). However the intervals during which plasma progesterone exceeded 64 and 32 nmol/l were significantly shorter in the treated bitches. The interoestrus interval was also significantly shorter (158 ± 16 days in bitches treated with aglepristone) than in the same group before treatment (200 ± 5 days), which is rather similar to what was previously described.
They concluded that administration of aglepristone during the early luteal phase in the non pregnant bitch affect progesterone secretion, but not sufficiently to shorten the luteal phase. The shortening of the interoestrus interval suggest that administration at this time influences the hypothalamo-pituitary-ovarian axis (Galac and al., 2004).
The results are contradictory with those of Polisca and al. (Polisca and al., 2010). In this study, aglepristone was administered at day 29 and day 30 after the estimated day of LH surge. *Administration of aglepristone during mid-luteal phase shortened the luteolytic phase*.
It is highly suspected that aglepristone may affect the gonadotrophin secretion of LH and FSH in a stage dependent way, as it is described for mifepristone in women and in rodents (Belido and al., 1999; Szabo and al., 2009).

This topic may create new fields of application of aglepristone in the coming years.

8.2. TREATMENT OF GROWTH HORMONE EXCESS IN DOGS

Growth hormone excess can lead to severe diseases in the canine species:
- *Acromegaly*, which is characterized by an important growth of soft tissues, bones and abdominal organs.
- Growth Hormone excess also promotes *diabetes*: it induces hyperglycemia, hyperinsulinemia and insulinoresistance state. Excessive growth hormone production can therefore alter glucose metabolic pathways and lead to diabetes mellitus.
- Growth hormone hypersecretion can also induce *corticotropic failure*.

These diseases are mainly affecting intact bitches and it was proved that *progesterone impregnation* was responsible of *excessive production of Growth Hormon at the mammary gland level*. Some other studies also showed that use of high doses of progestins can lead to the same symptoms (Selman and al., 1994). Indeed, clinical signs of acromegaly are usually observed during diestrus in the bitch, and diabetes is known to be more difficult to treat in bitches during this period.
Progesterone suppression lead to clinical cure (Eigenmann and Venker-Van Haagen, 1981).
Ovariectomy or arrest of progestin treatments makes growth hormone concentration return to normal. (82.9 ± 35.9 ng/ml before treatment to 2.8 ± 0.7 ng/ml after). All clinical signs disappeared in weeks to months. Therefore in bitches suffering from diseases related to excessive growth hormone production, the use of antiprogestins can be of great interest.
Bhatti and al. (2006) investigated whether aglepristone can be used as a treatment. Five beagle bitches were given medroxyprogesterone acetate to create an hypersomatotropism state. Then, they were administered aglepristone in a dose of 10 mg/kg SC once daily during two days, then once a week on day 8, 15 and 22 after beginning of treatment.
Treatment with *aglepristone significantly decreases the mean plasma concentration of Growth Hormone and IGF1*, which are the two main compounds responsible of acromegaly. Analysis of the pulsatile plasma profile showed a trend for a lower mean plasma Growth Hormone concentration one week after the last aglepristone treatment compared with these values before aglepristone administration.

In conclusion, *administration of aglepristone significantly decreases plasma Growth Hormone and IGF1 concentrations* in dogs suffering from hypersomatotropism, suggesting that it could be of great interest in bitches suffering from acromegaly (Bhatti and al., 2006).

8.3. ADJUNCTIVE TREATMENT IN NEOPLASIAS PRESENTING PROGESTERONE RECEPTORS

A clinical case was reported concerning the use of aglepristone in a bitch suffering from a *vaginal fibroma* (Rollon and al., 2008).

A 12 year-old entire, nulliparous crossbreed female dog presented with a history of vulvar bleeding, bulging of the perineum and faecal tenesmus. A firm, non painful perineal mass measuring 9.11 x 5.4 cm with erythema was detected, compatible with a vaginal tumor. Aglepristone was administered to the bitch on days 1, 2, 8, 15, 28 and 35 at the posology of 10 mg/kg. An incision biopsy was taken on day 15 and immunohistochemical analysis showed that the majority of neoplastic cells expressed progesterone receptors. Both the *cutaneous erythema and the faecal tenesmus had resolved* by day 28. A *50% reduction in size was observed by day 60* and surgical excision was at this moment easily performed. This study showed that *benign vaginal tumours of the dog that contains progesterone receptors can be reduced in size in a palliative or neoadjuvant setting using progesterone receptor antagonist aglepristone*.

Presence of progesterone receptors is reported in *tumors of the mammary glands* and of the genital tracts, and progesterone impregnation may sometimes enhance the growth, even if the role of progesterone in the mechanism remains uncertain and unclear (Geraldes and al., 2000).

In a recent study (Guil-Luna and al., 2010), aglepristone was administered at a dosage of 20 mg/kg SC twice at D1 and D7 in 22 non-spayed bitches in oestrus cycle phases. 5 bitches were used as control (untreated). Biopsy samples were taken prior to the first administration and removal surgery was done on day 15. Expression of Progesterone-Receptor (PR) and level of Proliferative Index (PI) and Apoptosis Index (AI) were measured. *Aglepristone had proved to reduce the PI and the AI in PR-positive canine mammary carcinoma*.

Antiprogestins may have an inhibitory effect on growth of neoplastic cells. In the woman, RU486 may have an antiproliferative effect on neoplastic mammary tumour cells, inhibiting mitosis and even having a cytotoxic effect on these cells (Horwitz, 1992). In rodents suffering from metastatic mammary tumours, treatment with mifepristone led to regression of lymphatic and pulmonary metastasis after 24 hours (Vanzulli and al., 2005).

One bitch suffering from meningioma was treated with antiprogestins during 6 weeks, but no evolution of the clinical signs was observed. Immunohistochemistry was performed, revealing the total absence of progesterone receptors on the tumors, making any interpretation difficult (Adamo and Cantile, 2003).

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