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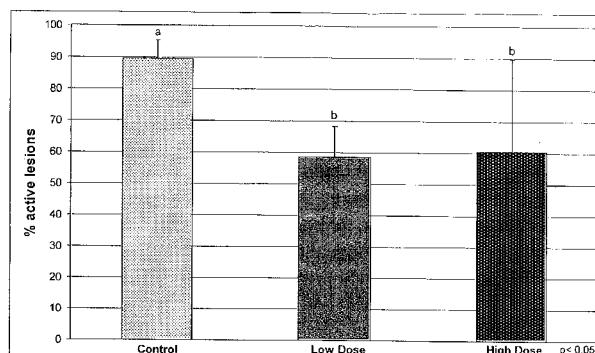
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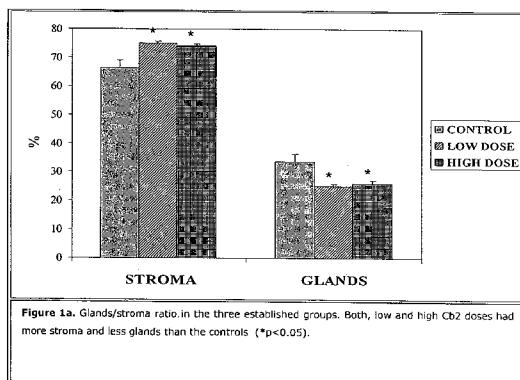
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[Continued on next page]

(54) Title: MEDICAMENT FOR THE TREATMENT OF ENDOMETRIOSIS



(57) Abstract: The use of a dopamine agonist in the manufacture of a medicament for the treatment or prevention of endometriosis.





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MEDICAMENT FOR THE TREATMENT OF ENDOMETRIOSIS

The present invention relates to medicaments for the treatment of endometriosis.

Endometriosis is a benign, chronic, gynaecological disease. It may be 5 defined as a presence of endometrial tissue, comprising both glandular epithelium and stroma, outside the uterine cavity. It is a benign gynaecological disorder, which, in a sub-population of female patients, may develop into an aggressive disease. Endometriosis is associated with various distressing symptoms including dysmenorrhoea, dyspareunia, pelvic pain and reduced 10 fertility.

It is known that angiogenesis (the process whereby new blood vessels are formed from pre-existing vessels) may be of importance in the development of endometriosis, and that vascular permeability factor/vascular endothelial growth factor (VP/VEGF) has a role in vascular genesis, and in both physiological 15 and pathological angiogenesis. The potential effectiveness of anti-angiogenic therapy for treating endometriosis has been assessed using a study using human endometrial tissues transplanted to immuno-compromised nude mice. Four different anti-angiogenic agents were administered three weeks after the endometrial explants had been transplanted (Nap *et al*, 2004). All four inhibitors 20 were able to reduce the size of established explants, and new blood vessel formation was stopped. However, the known anti-angiogenic agents are highly toxic, and rather difficult to introduce in a human clinical setting.

It has now been unexpectedly found that compositions which include a dopamine agonist may be used to treat endometriosis.

25 The present invention therefore provides the use of a dopamine agonist in the manufacture of a medicament for the treatment and/or prevention of endometriosis. The present invention also provides the use of a dopamine agonist in the treatment and/or prevention of endometriosis. Herein, the term "treatment of endometriosis" includes treatment to reduce (or remove) the 30 amount of endometrial tissue which is present outside the uterine cavity (e.g. reduction or removal of endometriotic lesions) and/or treatment to reduce and/or

ameliorate one or more symptoms associated with endometriosis (e.g. treatment to ameliorate and/or reduce the symptoms of dysmenorrhoea; treatment to ameliorate and/or reduce the symptoms of dyspareunia, and/or treatment to ameliorate and/or reduce pelvic pain). The term "treatment of endometriosis" 5 includes treatment to reduce the number of instances, and/or reduce the size of instances, of endometrial tissue which is present outside the uterine cavity (e.g. reduction of number and/or size of endometriotic lesions). The term "treatment of endometriosis" includes treatment to reduce the number of endometrial glands. The term "treatment of endometriosis" includes treatment which results in one or 10 more of: a significant decrease in the percentage of active endometriotic lesions; a significant loss of the cellularity and organisation manifesting characteristics of atrophic or degenerative tissue in endometriotic lesions; and a significant decrease in the number of new blood vessels in endometriotic lesions. The term "treatment of endometriosis" includes treatment to reduce the number and/or size 15 of endometriotic lesions on one or more of the ovary, the uterine cul-de-sac, the uterosacral ligaments, the posterior surfaces of the uterus, the broad ligament, the remaining pelvic peritoneum, the bowel, the urinary tract (including e.g. the bladder, and/or the ureters).

The term endometriosis includes, for example, peritoneal endometriosis, 20 ovarian endometriosis and deep endometriosis.

Herein, the term dopamine agonist means a compound that acts like dopamine, for example, a drug which interacts (e.g. binds specifically) with a dopamine receptor to mimic a dopamine action. It does not include compounds which are exclusively "dopaminergic substances", i.e. exclusively compounds 25 which, through different mechanisms of action, increase the levels of dopamine. The term dopamine agonist does not include the bridged indenopyrrolocarbazoles disclosed in US 6359130.

Dopamine agonists have previously been found to be useful in the 30 treatment or prevention of ovarian hyperstimulation syndrome (OHSS) (WO 2006117608). This is a condition where the capillaries increase their vascular permeability significantly. Dopamine agonists were found to be able to reverse this effect. However, the condition of endometriosis and the potential

effectiveness of anti-angiogenic therapy for treating endometriosis is not disclosed here.

Dopamine agonists within the terms of the invention include, but are not limited to, amantadine, bromocryptine, cabergoline, quinagolide, lisuride, 5 pergolide, ropinirole and pramipexole. A preferred dopamine agonist for use in the present invention is cabergoline. A preferred dopamine agonist for use in the present invention is quinagolide. The so-called "partial dopamine agonists" (e.g. terguride) may also be used in accordance with the invention. However, the use of dopamine agonists is preferred.

10 Preferably, a single dopamine agonist is used.

The dopamine agonist may be administered at a dose (e.g. an oral dose to a human patient) of between 25 micrograms per day and 80 mg/day, preferably between 50 micrograms per day and 5 mg/day, more preferably between 300 micrograms per day and 1 mg/day; suitable doses within this range 15 depend to the dopamine agonist to be used, as is readily apparent to those skilled in the art.

In a preferred embodiment, the dopamine agonist is cabergoline. Preferably the cabergoline is administered at a dose (e.g. an oral dose to a 20 human patient) of between 0.01 and 12.5 mg/week, preferably between 0.1 and 10 mg/week, more preferably between 0.5mg and 5 mg/week, more preferably at a dose of between 3.5 mg/week and 4 mg/week. The dopamine agonist may be administered as, for example, a single daily dose (of for example, between 0.1 mg/day and 5mg/day, from 0.2 mg/day to 1mg/day, for example 0.5 mg/day); or 25 the daily dose may be divided into two or more sub-doses to be taken at different times over a 24 hour period. The dopamine agonist (cabergoline) may be administered as a daily dose at the levels above, or as equivalent doses e.g. per week, twice a week, or every two days. In one regime, the dopamine agonist (for example, cabergoline) is administered at a total dose of between 3.5 and 30 12.5 mg per week (e.g. 4mg per week, 7mg per week, 10mg/week).

In another embodiment, the dopamine agonist is quinagolide. Preferably the quinagolide is administered at a dose (e.g. an oral dose to a human patient)

of between 25 and 1000 micrograms/day, preferably between 25 and 500 micrograms/day, more preferably between 25 and 300 micrograms/day. The dopamine agonist may be administered as, for example, a single daily dose; or the daily dose may be divided into two or more sub-doses to be taken at different times over a 24 hour period. The dopamine agonist (quinagolide) may be administered as a daily dose at the levels above, or as equivalent doses e.g. per week, twice a week, or every two days.

In another embodiment, the dopamine agonist is bromocryptine. 10 Preferably the bromocryptine is administered at a dose (e.g. an oral dose to a human patient) of between 10 to 80 micrograms/day, preferably 10 to 40 mg/day.

In one embodiment, the dopamine agonist is used as the only medical treatment for endometriosis. In other words, the dopamine agonist may be used 15 in the absence of other medical or surgical treatments [for example, in the absence of danazol].

In a further embodiment, administration of a dopamine agonist may be combined with other medical or surgical treatments for endometriosis [for 20 example, NSAIDs and/or hormonal treatments (danazol, OCs, medroxyprogesterone acetate, other progestins, GnRH agonists and antagonists, aromatase inhibitors)]. In a further embodiment, surgical treatment or medical treatment may be used prior, during or after treatment with dopamine agonist. These embodiments are discussed in a little more detail in the examples, below.

25

It has also been found that administration of dopamine agonist to a patient in need thereof may provide substantial clinical benefits such as, for example: significant decrease in the percentage of active endometriotic lesions; significant loss of the cellularity and organisation manifesting characteristics of atrophic or 30 degenerative tissue in endometriotic lesions; and significant decrease in the number of new blood vessels in endometriotic lesions.

Medicaments based on dopamine agonists have also the advantage of high dose tolerance, with safe and well documented clinical use records.

Further, it has been found that the dopamine agonist (for example, cabergoline) may be administered for long periods of time (e.g. 1 to 3 weeks (e.g. 1 to 21 days, for example 1 to 14 days), from 1 day to 3 months, 1 day to six months, 1 day to 12 months, or 1 day to 2 years, or longer) with therapeutically 5 beneficial effect, and low risk of side effects. The administration may be continuous at e.g. daily or weekly dose, or may be interrupted by one or more interruptions of, for example, a number (e.g. 1 to 3) of weeks or a number (e.g. 1 to 3) of months. The dopamine agonist may be administered for as long as pain (or other symptom) continues.

10 The dopamine agonist (for example, cabergoline, quinagolide) may be administered to a pregnant subject.

The treatment or prevention of endometriosis may be associated with a decrease in the amount of endometrial glands.

In an example of the invention, vascular endothelial growth factor (VEGF) 15 may be targeted by the dopamine agonist, as a factor in the development of endometriosis. The isoforms VEGF₁₂₁ and VEGF₁₆₅ appear to be mainly involved in the process of angiogenesis (Watkins, R.H., et al., Am. J. Physiol. 1999, vol. 276, pp. 858-67). Two specific endothelial cell membrane receptors for VEGF have been identified, VEGF receptor-1 (VEGFR-1; Flt-1) and VEGFR-2 (Flk- 20 1/KDR). The VEGFR-2 appears to be mainly involved in regulating angiogenesis and vasculogenesis.

VEGFR-2 (KDR) may thus be targeted by the dopamine agonist, as a factor in the development of endometriosis. Notch-4 may be targeted by the dopamine agonist, as a factor in the development of endometriosis. VEGF, 25 VEGFR-2 and Notch-4 may be targeted by the dopamine agonist, as a factor in the development of endometriosis. Other mechanisms of action of the dopamine agonist are within the scope of the invention.

The dopamine agonist is administered as a pharmaceutically acceptable preparation. Preparations may be administered in accordance with the invention 30 in pharmaceutically acceptable compositions that may optionally comprise pharmaceutically acceptable salts, buffering agents, preservatives and

excipients. Pharmaceutical preparations which include dopamine agonist(s) as active agents are well known in the art and are commercially available. For example, cabergoline is available under the registered trade marks Cabaser and Sogilen/Dostinex. The use of such commercially available dopamine agonist 5 preparations in the treatment of endometriosis is according to the invention.

The mode of administration selected will depend on the acuteness and severity of the condition being treated, and the dosage required. Any mode of administration that produces desired therapeutic effect without unacceptable adverse effects is relevant in practicing the invention. Such modes of 10 administration may include oral, rectal, topical, transdermal, sublingual, intramuscular, parenteral, intravenous, intracavity, vaginal, and adhesive matrix to be used during surgery. Various approaches for formulating compositions for use in accordance with the invention are described in the Handbook of Pharmaceutical Excipients, Third Edition, American Pharmaceutical Association, 15 USA and Pharmaceutical Press UK (2000), and Pharmaceutics – The Science of Dosage Form Design, Churchill Livingston (1988).

In a preferred embodiment, the administration is oral. Compositions suitable for oral administration include capsules, cachets, tablets, syrups, elixirs 20 or lozenges.

According to the present invention in a further aspect, there is provided a method of treatment or prevention of endometriosis comprising a step of administration to a patient in need thereof of a dopamine agonist.

Preferably the dopamine agonist is administered in the form of a 25 pharmaceutical preparation which includes one or more dopamine agonists as the active ingredient.

The dopamine agonist may be administered at a dose (e.g. an oral dose to a human patient) of between 25 micrograms per day and 80 mg/day, preferably between 50 micrograms per day and 5 mg/day, more preferably 30 between 300 micrograms per day and 1 mg/day; suitable doses within this range depend to the dopamine agonist to be used, as is readily apparent to those skilled in the art.

In a preferred embodiment, the dopamine agonist is cabergoline. Preferably the cabergoline is administered at a dose (e.g. an oral dose to a human patient) of between 0.01 and 12.5 mg/week, preferably between 0.1 and 5 10 mg/week, more preferably between 0.5mg and 5 mg/week, more preferably at a dose of between 3.5 mg/week and 4 mg/week. The dopamine agonist may be administered as, for example, a single daily dose (of for example, between 0.1 mg/day and 5mg/day, from 0.2 mg/day to 1mg/day, for example 0.5 mg/day); or the daily dose may be divided into two or more sub-doses to be taken at different 10 times over a 24 hour period. The dopamine agonist (cabergoline) may be administered as a daily dose at the levels above, or as equivalent doses e.g. per week, twice a week, or every two days. In one regime, the dopamine agonist (for example, cabergoline) is administered at a total dose of between 3.5 and 12.5 mg per week (e.g. 4mg per week, 7mg per week, 10mg/week).

15

In another embodiment, the dopamine agonist is quinagolide. Preferably the quinagolide is administered at a dose (e.g. an oral dose to a human patient) of between 25 and 1000 micrograms/day, preferably between 25 and 500 micrograms/day, more preferably between 25 and 300 micrograms/day. The 20 dopamine agonist may be administered as, for example, a single daily dose; or the daily dose may be divided into two or more sub-doses to be taken at different times over a 24 hour period. The dopamine agonist (quinagolide) may be administered as a daily dose at the levels above, or as equivalent doses e.g. per week, twice a week, or every two days.

25

In another embodiment, the dopamine agonist is bromocryptine. Preferably the bromocryptine is administered at a dose (e.g. an oral dose to a human patient) of between 10 to 80 mg/day, preferably 10 to 40 mg/day.

30

The administration of a dopamine agonist may be combined with other medical or surgical treatments for endometriosis [for example, NSAIDs and/or hormonal treatments (danazol, OCs, medroxyprogesterone acetate, other progestins, GnRH agonists and antagonists, aromatase inhibitors)]. In a further embodiment, surgical treatment or medical treatment may be used prior, during 35 or after treatment with dopamine agonist.

The applicants have found that the dopamine agonist (for example, cabergoline) may be administered for long periods of time (e.g. 1 to 3 weeks (e.g. 1 to 21 days, for example 1 to 14 days), from 1 day to 3 months, 1 day to six months, 1 5 day to 12 months, or 1 day to 2 years, or longer) with therapeutically beneficial effect, and low risk of side effects. The administration may be continuous at e.g. daily or weekly dose, or may be interrupted by one or more interruptions of, for example, a number (e.g. 1 to 3) of weeks or a number (e.g. 1 to 3) of months. The dopamine agonist may be administered for as long as pain (or other 10 symptom) continues.

The patient may be pregnant.

The treatment or prevention of endometriosis may be associated with a decrease in the amount of endometrial glands.

The present invention will now be illustrated with reference to the 15 Examples and attached drawings, in which:

FIGURE 1 shows the percentage of active lesions following the animal study discussed below, for the control group, and the groups treated with low dose (0.05 mg/kg/day) and high dose (0.1 mg/kg/day) of cabergoline; FIGURE 1a shows the glands/stroma ratio in the three established groups [both low and high 20 cabergoline ("Cb2") doses had more stroma and less glands than the controls (*p<0.05)].

FIGURE 2 shows the blood vessels (mm^3) for the control and low and high dose groups;

25 FIGURE 3 shows the percentage of "mature" and "newly formed" blood vessels in the animals of the control, low and high dose groups;

FIGURE 4 shows the Proliferation Index for the control and low and high dose groups;

30 FIGURE 5 shows the relative expression of VEGF, VEGFR-2 (KDR), Notch-4, Ang 1 and Wnt-1 for the control, low and high dose groups;

FIGURE 6a shows the presence of dopamine receptor 2 (Dp-r2) and VEGF in endometrial implants of the animals in the three established groups (control, low and high dose groups); and

FIGURE 6b shows the relative expression of VEGF, VEGFR-2 (KDR) and 5 D2R in endometriotic lesions (left-hand column) and in the endometrium (right-hand column).

Example 1

An experimental animal endometriosis model was developed in nude mice by inserting human endometrium fragments. Female mice (Hsd: Athimic Nude-10 nu, Harlan Ibérica S.L, Barcelona, Spain) were individually housed in autoclaved cages and bedding, in laminar flow filtered hoods. The animal room was maintained at 26 C with a 12-h light, 12-h dark cycle, and mice were fed *ad libitum* with autoclaved laboratory rodent chow and acidified water. All handling was performed in laminar flow filtered hoods. A mixture of 15 ketamine/medetomidine (75 µg/g ketamine and 1 µg/g medetomidine) (Ketolar®, Parke-Davis, España; Domtor®, Pfizer, Spain) i.p injected, was used to anesthetize mice before invasive procedures and atipamezole (Antisedan®, SmithKline Beecham, Spain) 1 µg/g i.p injected, to reverse the anesthesia effects, was used after invasive procedures, using sterile instruments.

20 At the age of 5 weeks, sterile 60-d release capsules containing 18 mg 17 β -estradiol (Innovative Research of America, Sarasota, FL) were placed sc in the neck of each animal. According to the manufacturer's information, capsules provide continuous release of hormone at serum concentrations of 150–250 pmol/liter, in the range of physiological levels in mice during the estrous cycle. 25 This stable physiological level of estrogen promotes the growth of transplanted human endometrium and avoids intermouse differences related to various stages of the estrous cycle.

30 Four days after insertion of the estrogen pellet, an entrance was made to the peritoneal cavity in the midline in the lower abdomen to insert fresh human endometrium from ovum pick-up donors. These fragments were stuck by glue (Vetabond®, 3M Animal Care products, USA) to the peritoneum. This protocol

allowed to mimic the pathologic situation of retrograde menstruation that occur in women with endometriosis.

Three weeks after implantation the animals were split into three groups. The first was a control group; the second was the low dose group, treated with 5 0.05 mg/kg/day oral cabergoline; and a high dose group, treated with an oral dose of 0.1 mg/kg/day cabergoline. Two weeks after treatment the animals were sacrificed and the endometriotic lesions sampled and analysed as set out below. The results are shown in Figs. 1, 1a and 2 to 6b.

The anti-angiogenic effects of the dopamine agonists were assessed by 10 by immunofluorescence, the antibodies used in confocal microscopy raised against Von Willebrand factor (vWF monoclonal IgG1, DAKO Corp., Denmark) present in endothelial cells and vascular smooth muscle cells (monoclonal IgG2 α -SMA-FITC conjugated de Sigma, St Louis, USA). Blood vessel which are not of new formation, are rounded by a smooth muscle layer which confers maturity 15 to them. Identification of endothelial cells was made employing vWF, whereas mature vessels were identified by α -SMA positive staining. Thus, vWF+ / α SMA- blood vessels were considered new or immature, while vWF+ / α SMA+ vessels were classified as old or mature blood vessels.

Morphometric study was performed to measure the implants area and 20 cellular density. Immunocytochemistry with Ki-67 (monoclonal IgG1 DAKO Corp., Denmark) antibody was performed in order to evaluate the proliferative activity of the implants. The histopathological and subcellular ultrastructural changes were detected using an optical microscope (OM), transmission electronic microscope (TEM) and histochemical staining. Optical microscopy (OM), transmission 25 electron microscopy (TEM) and morphometry were performed to ascertain the presence of endometrial glands and stroma and study subcellular ultrastructural changes, area of the implants and cellular density.

TaqMan Real-time PCR and $2^{-\Delta\Delta Ct}$ methods were used to analyze the gene expression profiles of three different markers that promote angiogenesis 30 (VEGF, VEGFR-2, Notch-4); Angiopoietin-1 (Ang-1), an antiangiogenesis marker which promotes the enlargement of existing vessels and resistance to leakage; and Wnt-1. Demonstration of the presence of the VEGF and dopamine type 2

receptor (Dp-r2) expression in experimental implants, human peritoneal endometriotic lesions, and endometrium, was conducted using the TaqMan Real-time PCR and $2^{-\Delta\Delta Ct}$ method (Fig 6a, 6b). Similarly, this method was used to demonstrate the presence of VEGFR-2 expression in human peritoneal
5 endometriotic lesions, and endometrium (Fig 6b).

Statistical analysis was performed using GraphPad Instat V3.0 (GraphPad Software, San Diego, CA, USA). The study was designed to detect differences in the different markers analyzed among treatment and control groups. Categorical data were expressed as number and percentage, and numerical data as mean \pm
10 standard error of the mean (SEM) except when specified. Significance was defined as $P<0.05$. One-way analysis of the variance was used for statistical analysis.

Toxicity

No mice in the study died following the administration of cabergoline.
15 Cabergoline did not appear to change the overall health of the mice, because the body of weight of the mice in the different treatment groups was not significantly different (results not shown).

Results

Figure 1 shows the percentage of active lesions following the animal
20 study discussed above, for the control group, and the groups treated with low dose (0.05 mg/kg/day) and high dose (0.1 mg/kg/day) of cabergoline. Animals treated with low (58.6 \pm 9.7%) and high (60.4 \pm 8.4%) doses, had a significant decrease ($p=0.0169$) in active lesions as compared to controls (89.6 \pm 5.7%). The animals treated with cabergoline (both low and high dose groups) have
25 significantly fewer active lesions when compared to the control group. In other words, treatment with cabergoline appears to reduce the number of active endometriotic lesions in this model. The OM and TEM studies showed that in the control group the endometriotic lesions presented a high cellular stroma and a histological aspect of complete reorganization and structure, as may be seen in a
30 typical human endometriosis lesion. However, in treated lesions (high and low dose of cabergoline) a lax stroma, with lost cellularity and organization, is

observed; this is characteristic of atrophic or degenerative tissue. The human endometrial stroma surrounding glandular areas, was easily differentiated from the muscular-conjunctive murine tissue.

These histological results were confirmed by morphometric analysis (Fig 5 1a). Although there was no statistical difference among groups in cellular density, there was a significant ($p=0.0093$) difference among groups in the ratio glands/stroma, as seen in Figure 1a; both low and high cabergoline doses had more stroma and less glands than the controls (* $p<0.05$). This indicates that cabergoline treatment may be associated with a decrease or reduction in the 10 amount of endometrial glands.

Figure 2 shows the blood vessels (mm^3) for the control, low and high dosage groups, divided between "mature" blood vessels and "newly formed" blood vessels. The control group has a greater proportion of newly formed blood vessels (indicative of significant angiogenesis), while the low and high dose 15 groups have a significantly greater proportion of mature blood vessels, suggestive of significantly reduced angiogenesis. This was also demonstrated by histology (results not shown). These results indicate that the cabergoline significantly reduced new blood vessel formation (angiogenesis), in this model.

Figure 3 shows the percentage of blood vessels in the control and low 20 and high dosage groups. The control group has approximately 74% of the total blood vessels as newly formed blood vessels, indicating significant angiogenesis. The low and high dosage groups, on the other hand, have approximately 85 to 89% of the total as mature blood vessels, indicating that angiogenesis is not significant. These results indicate that the cabergoline significantly reduced new 25 blood vessel formation (angiogenesis), in this model.

Figure 4 shows the results of a proliferation study. An immunocytochemistry study using the Ki-67 antibody (i.e. analysing the degree of cellular proliferation employing antibodies against Ki-67) was used to evaluate the proliferative activity of the implants using methods known in the art. Image 30 counting software was used to count the Ki-67 positive cells and hence calculate the proliferation index in each group. A significant ($P<0.001$) decrease in

proliferation was observed in lesions in animals treated with cabergoline (both low and high dose groups) compared with the control group.

The angiogenic status of the lesions was initially analyzed using immunofluorescence employing antibodies to identify new (vWF+/ α SMA-) and 5 old vessels (vWF+/ α SMA+) and confocal microscopy (Leica Confocal Software). Immunofluorescence employing antibodies raised against the Von Willebrand factor (vWF) present in endothelial cells and vascular smooth muscle cells (α -SMA) can be used to test the antiangiogenic action Cb2. Blood vessel which are not of new formation, are rounded by a smooth muscle layer which confers 10 maturity to them. Identification of endothelial cells was made employing vWF, whereas mature vessels were identified by α -SMA positive staining. Thus, vWF+ / α SMA- blood vessels were considered new or immature, while vWF+ / α SMA+ vessels were classified as old or mature blood vessels.

15 The implants presented a higher cellular stroma and the histological aspect of complete reorganization and structure typically seen in an endometriosis lesion in the control group, whereas the implants in mice included in the low and high dosage groups showed a lax stroma with loose cellularity and organization, which is characteristic of atrophic/degenerative tissue. Morphometry showed no difference in cellular density and stroma/glands glands area among 20 groups.

TaqMan Real-time PCR and $2^{-\Delta\Delta Ct}$ methods were used to analyze the gene expression profiles of three different markers that promote angiogenesis (VEGF, VEGFR-2, Notch-4); Angiopoietin-1 (Ang-1), an antiangiogenesis marker which promotes the enlargement of existing vessels and resistance to leakage; 25 and Wnt-1. The housekeeping 18S rRNA was used to normalize the target gene Ct values. The Ct value in each group was expressed relative to the control group Ct values (calibrator), to calculate the relative expression by the $2^{-\Delta\Delta Ct}$ method. cDNA obtained from sarcoma 180 tumor cells (S-180) and human umbilical vein endothelial cells (HUVEC) was used as negative and positive control, 30 respectively, for the VEGF and VEGFR-2 gene expression. Spleen was used as positive control for Ang-1 and Wnt-1, and lung was employed as a Notch-4 positive control.

Table 1 shows that the gene expression profiles of pro-angiogenic markers (VEGF, VEGFR-2 and Notch-4) were downregulated in the implants treated with low and high cabergoline ("Cb2") doses compared to controls. Ang-1 and Wnt-1 are considered to be anti-angiogenic markers; their expression profiles were upregulated in the implants of mice treated with low and high Cb2 doses compared to controls, showing that cabergoline treatment is associated with angiogenesis inhibition. The relative expression data from the table are shown graphically in Figure 5.

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Table 1: Angiogenic gene expression profiles

Angiogenesis marker	Gene	Control ±	Vehicle	LOW DOSE (0.05 mg/kg/day)	HIGH DOSE (0.1 mg/kg/day)	p value	Expression
+	VEGF	HUVEC	1.2±0.3	0.5±0.2	0.4±0.1	<0.05	<u>Downregulated</u> in Cb2 treated groups
+	VEGFR -2 (KDR)	HUVEC	1.0±0.1	0.4±0.1	0.3±0.1	<0.05	<u>Downregulated</u> in Cb2 treated groups
+	Notch-4	LUNG	1.0±0.1	0.5±0.1	0.4±0.1	<0.05	<u>Downregulated</u> in Cb2 treated groups
-	Ang-1	SPLEEN	1.1±0.2	3.2±0.6	3.7±0.6	<0.05	<u>Upregulated</u> in Cb2 treated groups
-	Wnt-1	SPLEEN	1.0±0.1	3.4±0.2	3.3±0.6	=0.07	<u>Upregulated</u> in Cb2 treated groups

Data expressed as Mean ± SD

The presence of dopamine type 2 receptors in human endometrial tissue and endometriotic implants in women undergoing surgery (laparoscopy) was 5 investigated. TaqMan Real-time PCR and $2^{-\Delta\Delta Ct}$ methods were used to ascertain the presence of dopamine receptor 2 ("Dp-r2", "D2R") and VEGF in human endometrial tissue, not only in implants (Fig 6a), but also in peritoneal endometriotic lesions obtained from 10 women at laparoscopy (Fig 6b – which also includes data for VEGFR-2). Positive and negative controls for Dp-r2 were 10 HUVEC and S-180 cDNA cells, respectively. In mice, the control Ct values were used as a calibrator, and in human peritoneal lesions the HUVEC Ct values were employed as calibrator to calculate the relative expression by $2^{-\Delta\Delta Ct}$ method.

Figure 6a shows the presence of VEGF and Dp-r2 in endometrial implants of the animals in the three established groups. There was a trend 15 towards increased Dp-r2 expression as the animals were treated with increasing doses of cabergoline. There is a trend towards decreased VEGF expression as the animals were treated with increasing doses of cabergoline.

Figure 6b shows (left-hand column) the relative expression of VEGF, VEGFR-2 (KDR) and D2R ("Dp-r2") in different types of endometriotic lesions; red, white and black. There is a clear difference in VEGF, VEGFR-2 and Dp-r2 expression according to the type of lesion.

5 Figure 6b also shows (right-hand column) the relative expression of VEGF, VEGFR-2 (KDR) and D2R ("Dp-r2") in the endometrium. The relative expression of VEGF and VEGFR-2 in the endometrium of subjects with endometriosis is higher than those without endometriosis; the relative expression of Dp-r2 in the endometrium of subjects with endometriosis is lower than those
10 without.

These results are shown in Table 2, below.

Gene	ENDOMETRIUM				ENDOMETRIOTIC LESIONS			
	WITHOUT ENDOMETRIOSIS	MODER- ATE	SEV ERE	P value	RED	WHITE	BLACK	P value
VEGF	1.0±0.1	2.2±0.7	2.1± 0.4	NS	17.8±5.8	10.1±4 .8	11.1±3.9	NS
KDR	1.0±0.1	2.1±0.6	1.9± 0.3	NS	47.4±15.0	26.9±1 2.2	23.6±7.2	NS
D2R	1.0±0.1	0.3±0.1	0.2± 0.1	=0.07	0.2±0.1	0.3±0. 2	0.6±0.3	NS

The results indicate that the dopamine agonist cabergoline, administered at a dose of 0.05 and 0.1 mg/kg/day, was able to:

20 (a) significantly decrease the number of active endometriotic lesions;
 (b) cause loss of cellularity and organisation presenting characteristics in atrophic or degenerative tissue in the endometriotic implants;
 (c) significantly decrease the number of new blood vessels in the endometriotic implants;
 (d) significantly decrease expression of markers of angiogenesis, and
25 cellular proliferation; and

(e) increase tissue degeneration and reduce the endometriotic implants.

There is a high homology between the human and rodent VEGF systems which offers an indication that the activity shown in the above rodent model is 5 applicable to human models. The results indicate that administration of dopamine agonists has a significant effect on endometriosis, possibly linked to action on angiogenesis. The results may be related to the presence of dopamine receptors in eutopic and ectopic endometrial tissue.

Example 2

10 A formulation as a tablet for oral use is 0.5 mg of cabergoline (commercially available as Dostinex®, Pfizer, Spain).

Further Examples

Example A: A patient undergoes diagnostic laparoscopy after suffering chronic pelvic pain and is diagnosed with endometriosis type III. At the same 15 laparoscopy, the patient undergoes surgery such as resection of available lesions and administration of cabergoline is initiated.

Example B: Patient diagnosed previously of endometriosis, presenting with symptoms of pelvic pain and dysmenorrhea. Administration of cabergoline is 20 initiated without surgery.

Example C: Patient diagnosed with endometriosis undergoing treatment with GnRH agonists (or danazol or aromatase inhibitors) and administration of cabergoline is initiated (with continued use of GnRH agonist) for a period. After 25 a further 3 or 6 months of no therapy, patient restarts cabergoline for a further period.

CLAIMS

1. Use of a dopamine agonist in the manufacture of a medicament for the treatment or prevention of endometriosis.
2. A dopamine agonist for use in the treatment or prevention of endometriosis.
3. The use according to claim 1 or claim 2, wherein said dopamine agonist is one of amantadine, bromocryptine, cabergoline, quinagolide, lisuride, pergolide, ropinirole and pramipexole.
4. The use according to any preceding claim wherein the dopamine agonist is cabergoline administered at a dose of between 0.01 and 12.5mg/week.
5. The use according to any preceding claim wherein the dopamine agonist is quinagolide administered at a dose of between 25 and 1000 micrograms/day.
6. The use according to any preceding claim wherein the dopamine agonist is bromocryptine administered at a dose of between 10 and 80mg/day.
7. The use according to any preceding claim wherein the dopamine agonist is administered for a period of between 1 day and 2 years.
8. The use according to any preceding claim, wherein the dopamine agonist is administered in combination with other surgical or medicinal treatment for endometriosis.
9. The use according to any preceding claim wherein the dopamine agonist (s) is used for the treatment or prevention of endometriosis in a pregnant subject.
10. The use according to any preceding claim, in which vascular endothelial growth factor (VEGF) is targeted.
11. The use according to any preceding claim in which VEGFR-2 is targeted.
12. The use according to any preceding claim in which Notch-4 is targeted.
13. The use according to any preceding claim in which the treatment or prevention of endometriosis is associated with a decrease in the amount of endometrial glands.
14. The use according to any preceding claim in which the treatment or prevention of endometriosis is associated with reduction (or removal) of an amount of endometrial tissue which is present outside the uterine

cavity; and/or treatment to reduce and/or ameliorate one or more symptoms associated with endometriosis.

15. The use according to any preceding claim wherein the administration is oral, and in the form of compositions as capsules, cachets, tablets, 5 syrups, elixirs or lozenges.
16. A method of treatment or prevention of endometriosis comprising a step of administration to a patient in need thereof of a dopamine agonist.
17. A method according to claim 16 in which the treatment or prevention of 10 endometriosis is associated with reduction (or removal) of an amount of endometrial tissue which is present outside the uterine cavity; and/or treatment to reduce and/or ameliorate one or more symptoms associated with endometriosis.

Fig 1

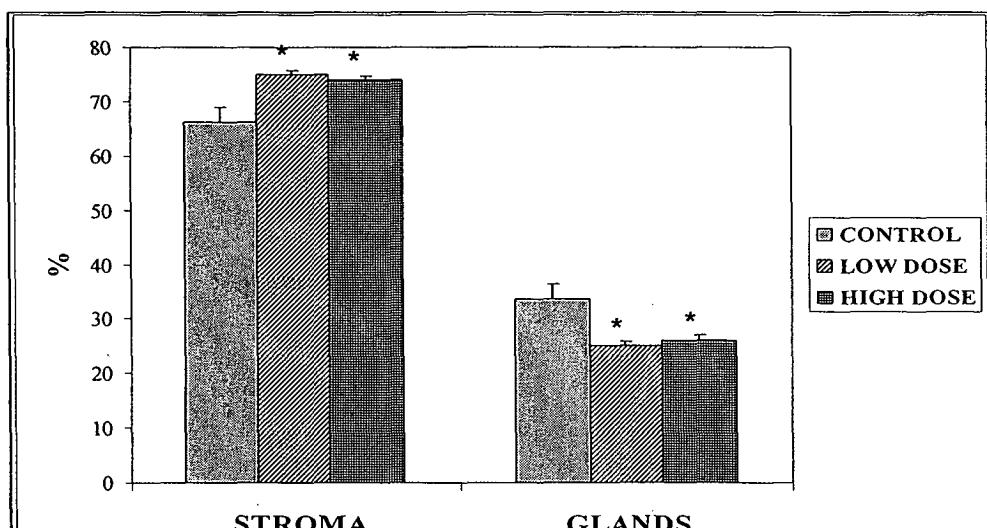
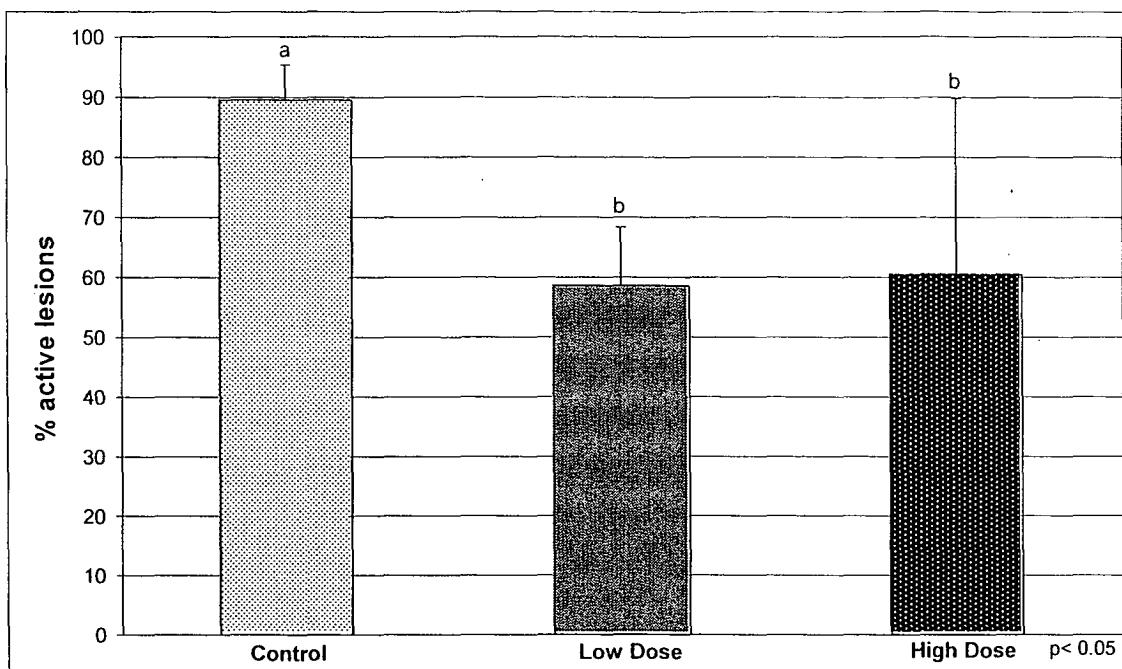


Figure 1a. Glands/stroma ratio in the three established groups. Both, low and high Cb2 doses had more stroma and less glands than the controls (*p<0.05).

Fig 2 (Angiogenic Study)

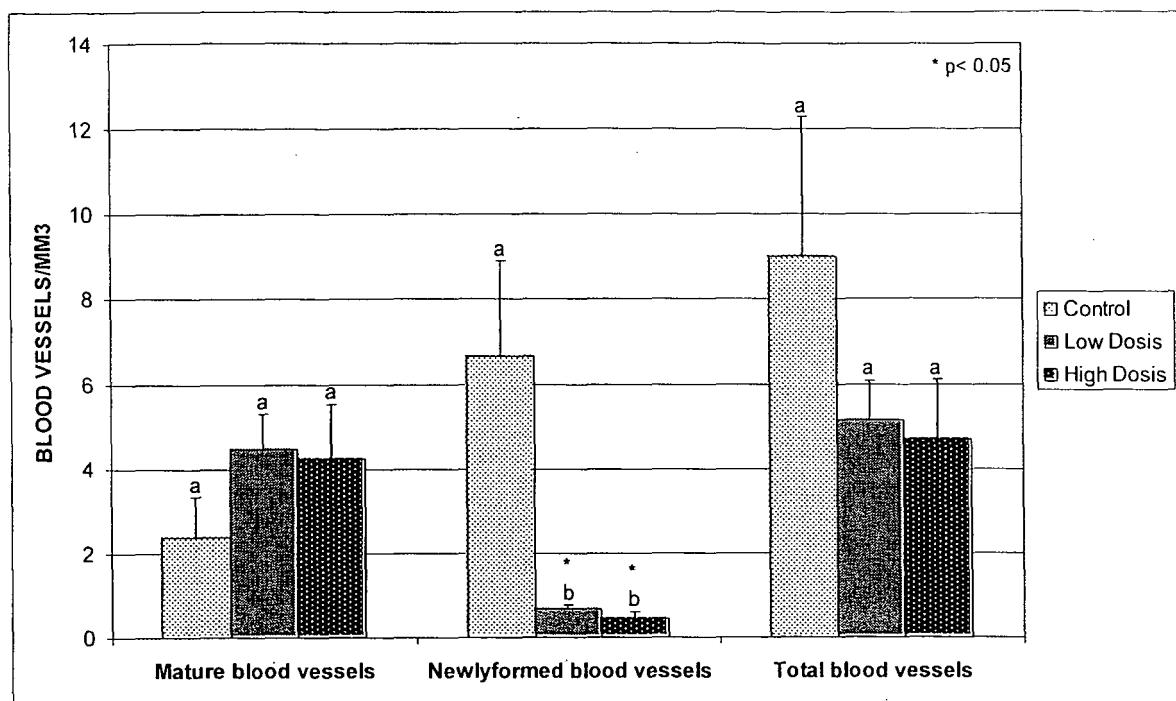


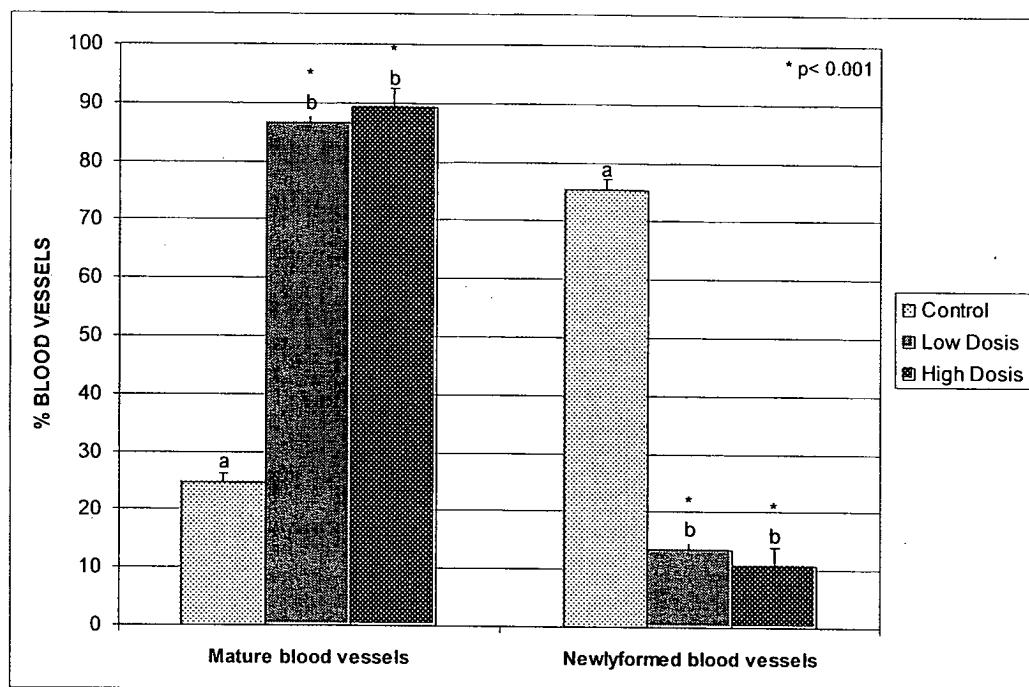
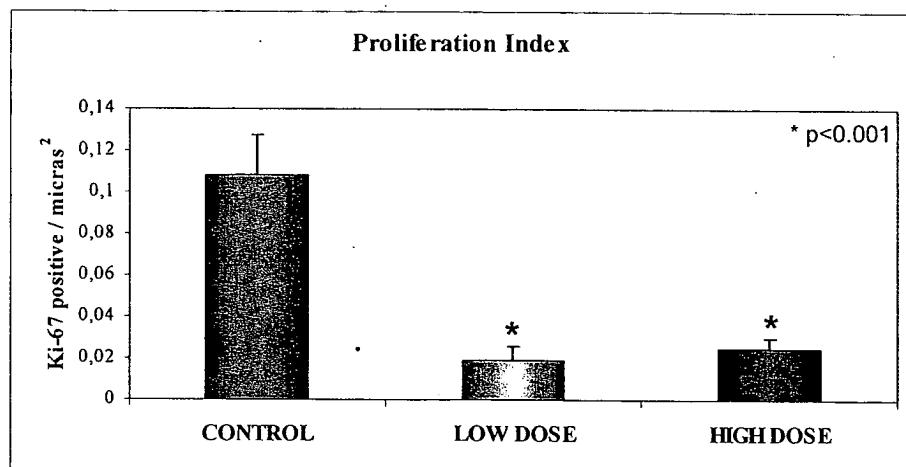
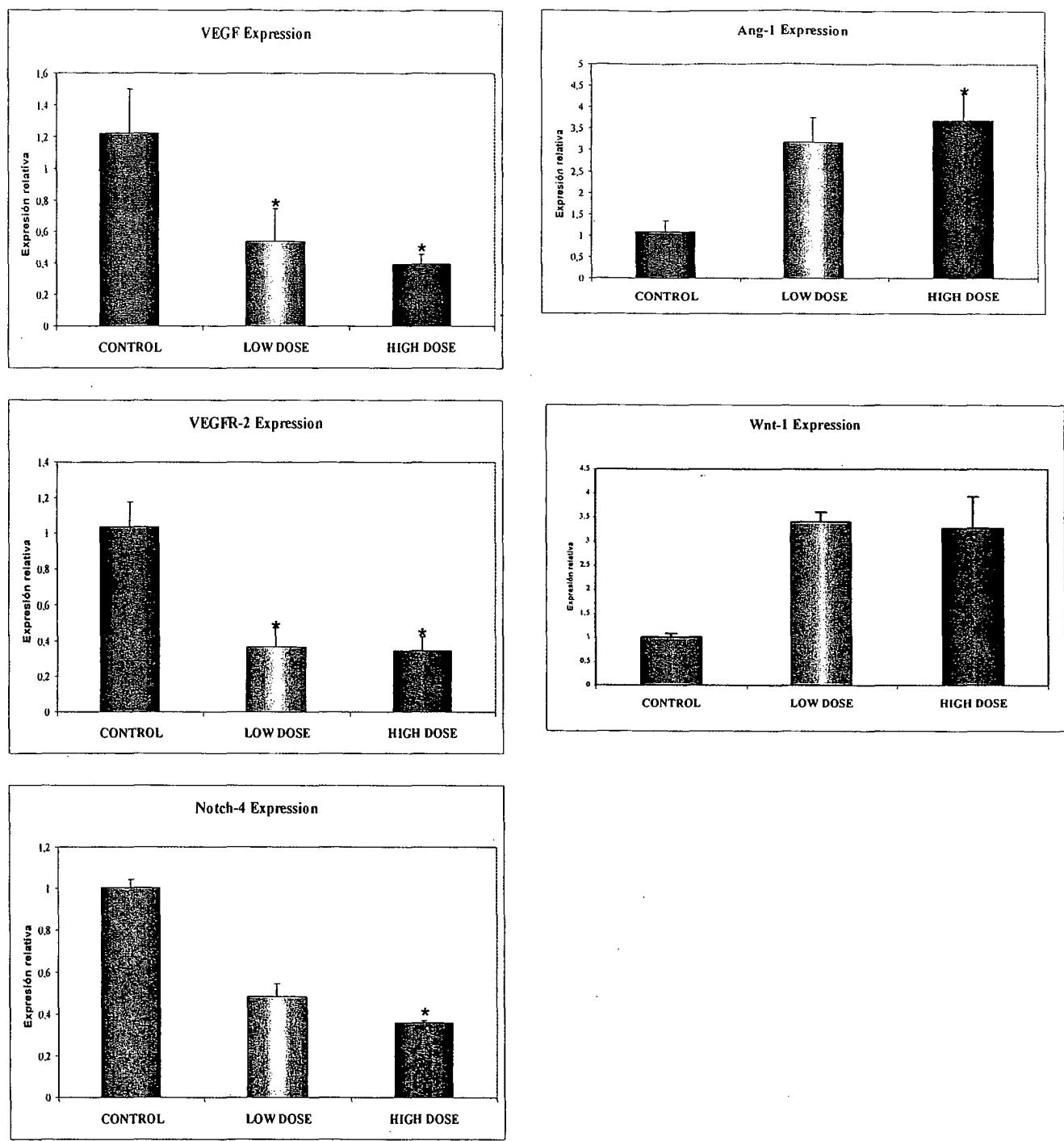
Fig 3 (Percentage)

Fig 4: Proliferation Study

**Fig 5**

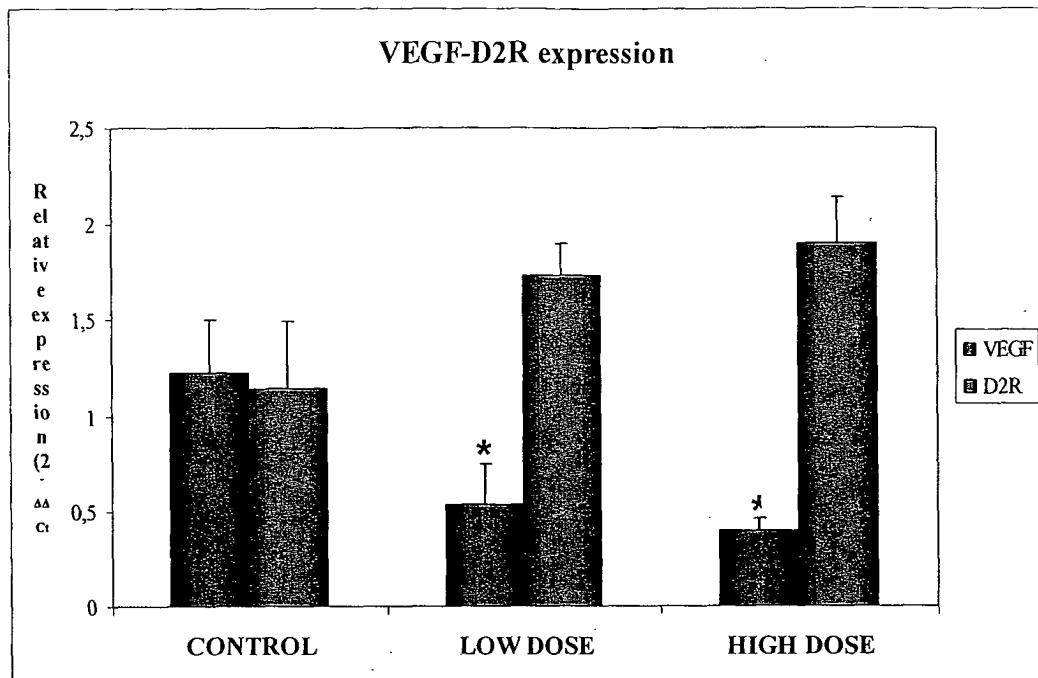


FIGURE 6a shows the expression profile of dopamine receptor 2 (Dp-r2, "D2R") and VEGF in endometrial implants of the animals in the three established groups (control, low and high dose groups)

Fig 6b

