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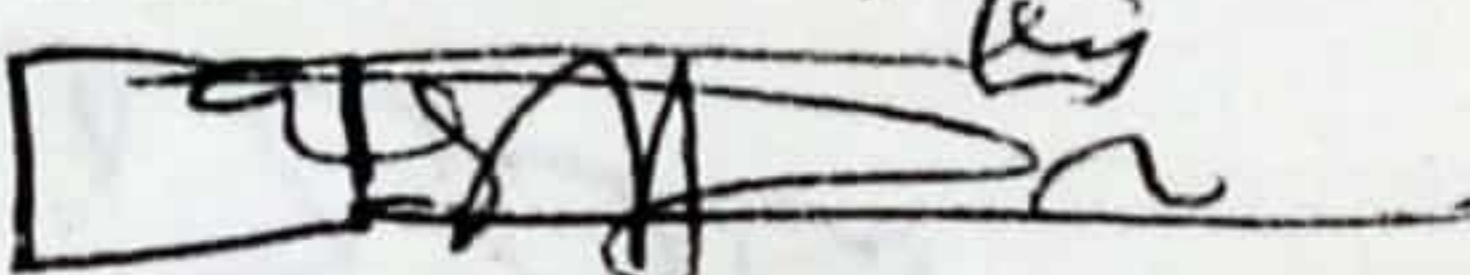
**DEPT. OF BIOLOGICAL SCIENCES
KWAME NKRUMAH UNIVERSITY
OF SCIENCE & TECHNOLOGY
KUMASI**

**THE EFFECT OF AN ALCOHOLIC EXTRACT OF FRUITS OF *Jatropha curcas*
ON SOME OF THE REPRODUCTIVE PROCESSES OF THE FEMALE
ALBINO RAT (*Rattus norvegicus*)**

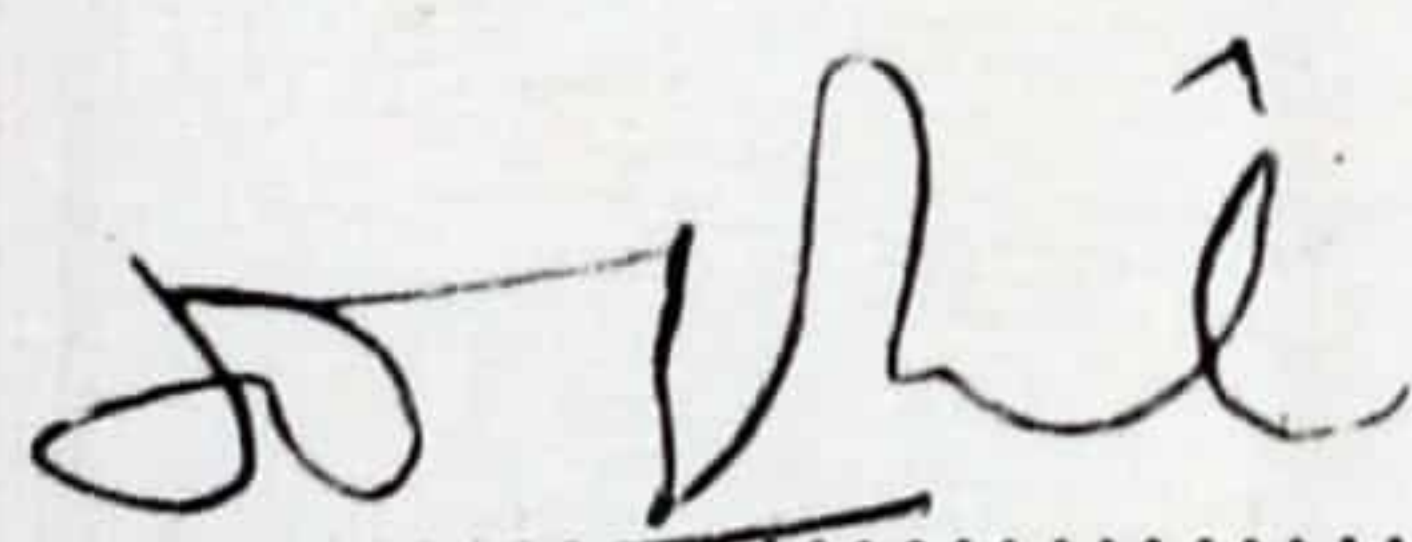
THE EFFECT OF AN ALCOHOLIC EXTRACT OF FRUITS OF *Jatropha curcas* ON
SOME OF THE REPRODUCTIVE PROCESSES IN THE FEMALE ALBINO RAT
(*Rattus norvegicus*)

A THESIS
PRESENTED TO THE KWAME NKRUMAH UNIVERSITY OF SCIENCE AND
TECHNOLOGY, KUMASI. GHANA.
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
MASTER OF SCIENCE (REPRODUCTIVE BIOLOGY) DEGREE

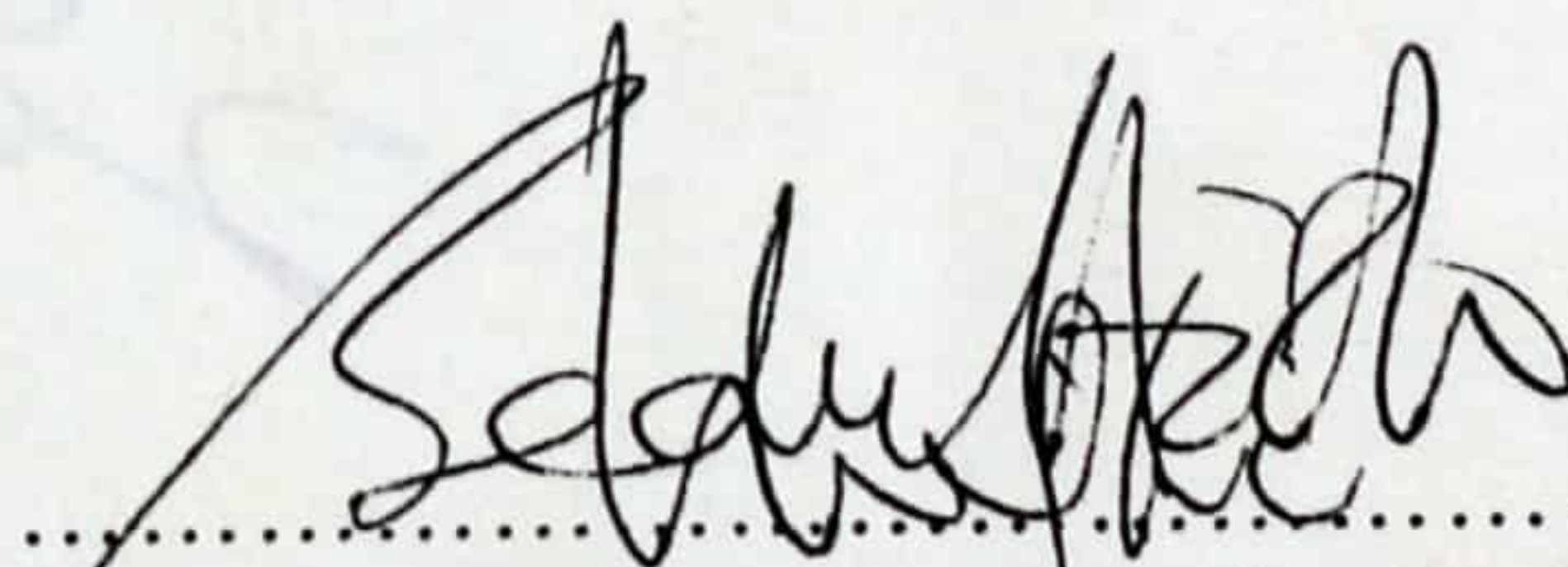
BY
JAMES AFRIYIE
SEPTEMBER, 2000



.....
CANDIDATE



.....
SUPERVISOR (Dr. Kofi Owusu-Daaku)



.....
SUPERVISOR (Mr. Kweku Bedu-Addo)

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ACKNOWLEDGEMENTS

My thanks go to God the All-powerful who sustained me with his grace and power throughout the entire of the project.

I am also grateful to my project supervisors, Dr. Kofi Owusu-Danku of the Biological Sciences Department and Mr. Kwaku Bedu-Addo of the School of Medical Sciences for their constructive criticisms and review of the synopsis and the work of this project.

I want to thank my lecturers whose knowledge I used to write up this project. They include Professor P. A. E. Addy, Dr. E. T. Abagyanaga and Mr. Kwaku Bedu-Addo all of the School of Medical Sciences. Professor A. C. Sackeyfio of the Faculty of Pharmacy. Professor Osei and Professor Gyavuu both of the Arts and Sciences Department. Mr. Woyome of the Social Sciences Faculty and Mr. Gyana of the Department of Mathematics.

I am also grateful to the programme co-ordinator, Dr. Lawson, the head of Department of Biological Sciences, Dr. E. Frempong and the Dean and members of the Board of Post-graduate Studies for their patience and tolerance during the period of the project.

DEDICATED TO

I am grateful to Dr. Seth Osei and Mr. Osei Ampomah who helped me in the statistical analysis. Mr. Sefa and Mrs. Agatha Sarfo-Gyamfi deserve credit for helping me to write this report on time. Mr. Kofi Nkoro, Mr. Nso, Mr. G. K. Kusi and the other technicians who assisted me in the laboratory should also be mentioned.

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ABSTRACT

Jatropha curcas is reported to be one of the many herbs used to terminate pregnancy in Ghana. Notwithstanding the folkloric claim that *Jatropha curcas* is an effective abortifacient herb, there are also wide speculations about the adverse effects of the herb on the users including death.

The present work investigated the effect of an alcoholic extract of fruits of *Jatropha curcas* on some of the reproductive processes on the female albino rat. The alcoholic extract of fruits of *Jatropha curcas* at different dose levels of 2.5, 2.0, 1.5 and 0g/kg were orally administered to female rats in three different experiments. Each of the experiments involved thirty-two (32) female rats.

The first experiment determined the effect of the extract on the length of oestrous cycle, development of ova and ovulation in the albino rat. In this experiment, the rats were given their required dosages of the extract for 16 days. Vaginal smear patterns of the rats were examined during the period of the experiment. At the end of the administration period, the rats were sacrificed and the number of corpora lutea in the ovaries was counted. Histological sections were also made of the ovaries for examinations.

The second experiment determined the effect of the extract on positive mating and implantation. In this experiment, the animals were given their required dosages of the extract for 5 days before they were paired with males. The number of positive mating (pregnancies) was counted and recorded. The administration of the extract continued for the pregnant rats up to the 5th day of pregnancy. At the end of the administration period, the animals were sacrificed and the number of implantation sites in the animals were

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counted and recorded. Histological sections were made from the uteri of the animals in the different groups for examination.

The third experiment determined the effect of the extract on ova wastage or foetal loss (abortion). In this experiment, the pregnant rats were given their required dosages of the extract starting from day 6 of pregnancy up to day 16 of pregnancy. The animals were sacrificed at the end of the administration period and the number of corpora lutea, and number of normal foetuses removed were counted and recorded. The number of ova wasted was calculated.

The data obtained showed that the concentrations of the alcoholic extract of fruits *Jatropha curcas* used, did not affect the length of oestrous cycle, the development of ova and ovulation in the albino rat. This is because the hypothalamo-pituitary-gonadal hormone secretions, on which the above processes depend, were not affected by the extract.

The extract did not also affect positive mating, in the rat. The extract had a significant reduction effect on implantation and also caused foetal loss (abortion) in the rats. It probably interfered with uterine contraction and mitosis in the developing foetuses. There was also loss of weight in the female rats, which were given the extract.

The fruits of *Jatropha curcas* used therefore showed to be abortifacient in albino rats.

CHAPTER ONE

INTRODUCTION

The knowledge and use of plants as a source of drug is an old phenomenon worldwide. The earliest written records of medicine date back about 3,500 years, but paleontologists have found bunches of medicinal herbs among the fossilized remains of our Neanderthal ancestors (Polunin and Robbins, 1992).

Surviving Egyptian papers from around 1,600 BC list a wealth of plants, animals and minerals remedies including many still used today. Examples of those listed include plants such as coriander, fennel, gentian henbane, juniper, pomegranate, senna, thyme and wormwood. The Old Testament of the Holy Bible of Christians is also rich in references to medicines from nature such as mandrake, cinnamon, frankincense and asafetida (Polunin and Robbins, 1992).

Marian Treben, a world-renowned herbalist and author of the International best seller 'Health from God's Pharmacy's has provided one of the most complete and accessible guide to herbal remedies that are available today. Marian in her own words says 'there is a plant for every illness' (Treben, 1980).

About 50 percent of all drugs preparations in the industrialised countries are derived from natural products. Besides the pure substances of natural origin, there is also the potential for the industrial development of traditional herbal remedies and plant extracts for treatment or prevention of diseases (Bruhn, 1989).

For the past several centuries, our ancestors have practiced traditional medicine using different species of plant and animal materials for the treatment and healing of diseases. These practices still go on among the 70-80 percent of rural population of Ghana who have no access to allopathic doctors or hospitals. Even in the urban centres and cities many people travel long distances for what is called

traditional or native medicine before they consider themselves fully cured (Ampofo, 1992)

In Ghana, a pharmacopoeia has been prepared to help make information on medicinal plants that have been investigated scientifically available to researchers. There are 50 of the most commonly used medicinal plants contained in the first edition of the Ghana Herbal Pharmacopoeia (Ampofo, 1992). *Jatropha curcas* is however not among the listed plants.

The sticky sap of *Jatropha curcas* is used to cure sores on the tongues of babies. The root-barks, when dried and pulverized can be used for dressing sores. The leaves are (dangerous) purgative and are given as a remedy against diseases such as rheumatic pains, and sores (Dalziel, 1937).

Jatropha curcas contains a non-drying fixed oil consisting mainly of glycosides of stearic, palmitic, oleic and curcinoleic acids. The fruits and seeds are thought to contain in addition, a contraceptive principle (Mameesh, *et al.*, 1963).

A significant number of young women in Ghana especially, those in the rural areas are alleged to use *Jatropha curcas* as an abortifacient herb. Whereas some claim that *Jatropha curcas* is effective in abortion in women, many are also reported to die from attempted abortion with the herb. Unsafe abortion is a major cause of death of women all over the world. About one out of every 150 abortions results in the woman's death (WHO, 1994). An estimated 3.7 million unsafe abortions are performed each year in sub-Saharan Africa (WHO, 1994). The most common abortion complications are incomplete abortion, sepsis, hemorrhage and intra-abdominal injury (Mati *et al.*, 1984). Except for intra-abdominal injury, all complications can result from either spontaneous abortions (miscarriage) or induced abortion. Left untreated each can lead to death (Kamau, 1990). Also women

surviving immediate abortion complications often suffer life long disability or face elevated risk of complications in future pregnancies (Herz and Measham, 1978)

Herbs, drugs or caustic chemicals swallowed or placed into the vagina or cervix can cause toxic reactions and lead to hemorrhage. It has been reported that pieces of the stem of *Jatropha curcas* inserted into the vagina of pregnant women to cause abortion. Others prepare decoction of *Jatropha curcas* and other herbs and swallow them. It is generally known that herbs in their crude form have both curative and lethal effects on man (Sarpong, 1985). The indication for the use of various medicinal plants is usually not well defined for several unrelated disorders. Also poisoning can occur because of misidentification of a plant or the unknown or ignored toxicity of a correctly identified plant (Bedu-Addo, 1993). The importance of researches into the therapeutic properties of traditional medicinal plants, their toxicity and standard dosages cannot be over emphasized.

The work of Mameesh and others in 1963 showed that *Jatropha curcas* fruits when used to feed rats can make them infertile. Other workers have also demonstrated the abortifacient property of different extracts of fruits of *Jatropha curcas*, when administered orally to the pregnant rats. (Goonasekera *et al.*, 1995). Despite the fact that ethnomedical information refers to the use of *Jatropha curcas* for control of reproduction, laboratory studies on the effect of *Jatropha curcas* on pregnancy appear to be limited to two studies by Mameesh *et al.*, (1963) and Goonasekera *et al.*, (1995). After their research, Goonasekera and others called for further studies to be undertaken on *Jatropha curcas*. Furthermore, documented information on the scientific investigation on *Jatropha curcas* in reproduction is not available in Ghana, and whereas some people in Ghana claim that *Jatropha curcas* is an efficient abortifacient herb, others completely disagree and rather refer to the herb

as a 'Killer herb'. It is against this background that this research was carried out to find out whether an alcoholic extract of *Jatropha curcas* collected from Ghana and intragastically administered to female rats would cause similar abortion complication or alter any of its reproductive processes, as reported elsewhere.

If the present work is able to establish some standard properties of *Jatropha curcas* as an anti-fertility herb, the cost will be within the means of Ghanaians. It will also be a more affordable drug than the orthodox drugs and could be prescribed by traditional practioners in the form of a crude extract or a semi purified isolate.

This work therefore aims at investigating the abortifacient and other properties of an extract of *Jatropha curcas* in female albino rats including its effects on

- i. Oestrous cycle length
- ii. Development of ova
- iii. Ovulation
- iv. Positive mating (pregnancy)
- v. Implantation
- vi. Ova wastage or foetal loss (abortion)

CHAPTER TWO

2.0 LITERATURE REIVEW

2.1 *JATROPHA CURCAS*

Jatropha curcas commonly called physic Nut or Barbados Nut belongs to the family Euphorbiaceae and the genus *Jatropha* (Mameesh *et al.*, 1963). From Greek, it means a physician and this refers to the medicinal properties of the plant. It is also sometimes called Poison Nut and Purging Nut. In Ghana *Jatropha curcas* is called by different names by the different tribes and clans. It is called 'Aborototo' in Twi, 'Nkrangyedua' in Akyem, 'Nkaneadua' in Asante, 'Adaadze' or 'Aborokyraba' in Fante, 'Taplekae' in Nzema, 'Kuadidi' in Ada, 'Kplukatso' in Ga, 'Babatsi' or 'kpoti' in Ewe (Anto, 1991).

2.1.1 Morphology and Botanical Character

Jatropha curcas is a shrub or that could grow up to 6metres high. The leaves, which are 3 -5 lobed, occasionally entire and glabrous, measure 10 – 15 centimetres inches by 7 – 13 centimetres. They are broadly ovate, cordate and acute. The petiole is about 7 – 22 centimetres inches long and the stipules caducous. Flowers, which appear yellow-green, are in cymose panicles and the petals united in the middle. Stamens are about 10 with the five inner connate half- way up. The capsule is about 1 inch long, subglabose or ellipsoid. Seeds that are three in number are ovoid - oblong, dull brownish - black in colour and about 2 centimetres long. (Anto, 1991)

2.1.2 Distribution

Jatropha curcas, even though known to be an American plant, is widely spread in the tropics. It is believed to have been brought to Africa and the East by the Portuguese for cultivation (Goonasekera *et al.*, 1995). It is a native of Mexico though now grows in tropical countries. In Ghana *Jatropha curcas* is commonly grown for hedges and fences in some villages. It is found in most parts of the country (Anto, 1991).

2.1.3 Constituents

Jatropha curcas contains a non drying fixed oil consisting mainly of glycerides of stearic, palmitic oleic and curcinoleic acids (Mameesh *et al.*, 1963). According to Irvine (1961), the oil of the seeds of *Jatropha curcas*, known as curcas consists of about 29 -40% fixed oil and it is made up of esters of palmitic and stearic acid (10 - 17%); oleic acid (45 - 62%); Linoleic acid (18 - 47%); myristic acid and arachidic acids ((less than 0.1%).

It has been shown that the seeds owe their purgative property to the oils (Irvine, 1961). The oils also contain a toxic principle, which is resino-lipoid in character.

Besides the fatty oil seed kernels, *Jatropha curcas* contains phytosterols, a ptiytosterolin (a glycoside), large amounts of sucrose and resinous matter, having nauseating, purging and griping effects (Anto, 1991). The fruits and seeds are thought to contain a contraceptive principle (Mameesh, *et al.*, 1963). It is believed that Jatrophone cytotoxic agent is also a constituent of *Jatropha curcas* (Farnsworth *et al.*, 1975).

Jatropha curcas contains abundant sap, cloudy in appearance and soapy to touch, believed to be due to the presence of saponins. It contains 10% tannins. A third poison, a resin (crotin resin) occurs in the seeds and causes redness and pustular eruptions of the skin. The bark contains a wax composed of a mixture of melissyl alcohol and its melissinic ester (Irvine, 1961)

2.1.4 Uses of *Jatropha curcas*

Jatropha species in general find use both domestically and medicinally by the indigenous people in every region where it is found (Anto, 1991)

The importance of plants as a source of antifertility drugs had been emphasised by many researchers (Chaudhury, 1986; Farnsworth *et al.*, 1975). *Jatropha curcas* is used to cause abortion in Ghana. A decoction of the dried roots with some ginger is administered as an enema or a young stem is cut and one end sharpened. The sharpened end is inserted into the cervix for some few hours and this causes abortion (Anto, 1991).

Jatropha curcas plant are often planted in graveyards in Africa and used as hedges in most villages in Ghana (Anto, 1991). The seeds, ground and mixed with palm oil are used in Gabon to kill rats while in Ghana the dried leaves are burnt and used to fumigate houses against bed bugs. The seeds are sometimes added to *strophanthus* seed to make arrow poison (Irvine, 1961).

The leaf decoction (0.10g to a liter of water) is used as a diuretic or causes bilious vomiting (Kerharo and Bouquet, 1950).

Jatropha curcas has a lot of medicinal uses. A lotion made from the crushed leaves in hot water is widely used in treating Guinea worm sores, or sores (Dalziel, 1937). The boiled leaves, mixed with lime are drunk or used for round worms. The

leaf decoction is drunk or used for roundworms. The leaf decoction is also drunk for jaundice (Irvine, 1961).

2.2 THE ALBINO RAT (*Rattus Norvegicus*)

The information obtained from other animals help to elucidate processes taking place in man. Direct extrapolation from one species to another is rarely possible but for most biological processes there are animal models which provide very useful information of direct relevance to man (Finn, 1980).

The albino rat, scientifically called *Rattus norvegicus* is probably the most suitable test animal for many aspects of physiological studies. The Rats has been used in practically all fields of investigation (Farris, 1957). By a fortunate coincidence a rat shows a similarity to man in many of its physiological and medical processes (Rosevear, 1969). Baker and others (1979) reported that studies done so far with the rat have indicated that it (the rat) responds to almost all-potential drugs in a way, which is markedly similar to other mammals.

Albino rats are gentle, clean, easily handled, readily trained and inexpensive to maintain. They live approximately for three years and begin breeding at about three months of age. The laboratory rat attains puberty earlier than its wild counterpart, and it is more prolific (Robinson, 1979) Puberty occurs at 50 to 60 days of age (Baker *et al.*, 1979).

Careful observations have shown that the gestation period of the albino rat is usually between 21 and 23 days. Gestation however may be extended for a week or more, if a female is carrying a large number of young and suckling a previous litter (King, 1913) The litters are large and can be secured at any season. Fertility is at its maximum when the rat is between 100 to 300 days of age (Farris, 1957).

2.3 THE MAMMALIAN REPRODUCTIVE PROCESSES

Reproductive physiological processes take place in the reproductive systems of mammals. Probably the most important organs in the reproductive system of female mammals are (1) the ovaries in which the development and liberation of the ova or eggs take place, and (2) the uterus or womb in which processes like implantation, development of the embryo and ova wastage take place. The development and liberation of ova is normally controlled and recurs in a cycle termed the oestrous cycle.

2.3.1 THE OESTROUS CYCLE

The non-breeding season or period of rest in a female mammal, when the ovaries and accessory reproduction organs are quiescent (at least relatively) and the animal shows no disposition to seek a mate is called the anoestrus period or simply anoestrus. Its close marks the beginning of the sexual season, and generally a change in the metabolism not only of the gonads and reproductive tract but also of the endocrine organs and the body generally. The sexual season is characterised by a repetition of a cycle of activities. The cycle of activities is more or less continuous and the end of one cycle is followed immediately by the start of the next (Tooles and Tooles, 1991). In human females, the onset of the first oestrous (menstrual) cycle is called menarche and represents the start of puberty. This takes place around the age of 12 years although the age varies between individuals. Two major events, which alternate in the cycle, are ovulation and oestrous (menses). Ovulation is an event that recurs at regular intervals during the reproductive period of a sexually mature female. The word oestrous or oestrus is a Latin adaptation of a Greek term. This is defined by the Oxford English Dictionary as something that stings or goads one on, a

stimulus, vehement impulse, frenzy, and its specific biological meaning is the "Sexual orgasm" and "rut of animals" (Eckstein and Zukerman, 1962).

Oestrus is like a baptism, it is an outward and visible sign of an inward and invisible event, in this case ovulation. It is a behavioural strategy to ensure that the female is mated at the time of ovulation (Short, 1984). At oestrous, the female does not only become highly attractive to the male at this time, but she is also receptive to his advances and may even actively seek him out, a behaviour known as proclivity (Short, 1984).

Animals in which oestrus occurs once during sexual season are called monoestrus animals. Those in which several oestrus cycles occur are called polyoestrus animals.

The first of the sexual season is occupied by proestrus and it is characterised by marked changes in the generative organs. The lining of the uterus grows and becomes congested. Proestrus is the period often referred to by breeders as the time when a female animal is coming "on heat" or "coming in season".

The second period, oestrous marks the climax of the process. It is during oestrus, that the female is willing to receive the male and fruitful coition is rendered possible in most animals. The periods of proestrus and oestrus are commonly referred to together as the heat or "Brust period" (Bedu-Addo, 1993).

If fertilisation takes place as a result of coition during oestrous, gestation follows. If it does not occur, oestrus is succeeded either by a short recovery period called metoestrous during which oestrous changes in the generative system subside.

In some animals, e.g. the rat, metoestrus is succeeded by only a short interval of quiescence. This short interval which sometimes lasts for only a few days is called dioestrus. Dioestrus in turn is followed by another proestrus period. Ovarian cycle

length is species-specific. Whether marked by periodic onset of menses (Menstrual cycle) or oestrus (oestrous cycle), it involves the same sequence of ovarian changes; preovulatory follicular maturation, ovulation and functional life span of the corpus luteum (Hillier, 1985). During the oestrous cycle, change occur in the vagina. During oestrus, the vaginal epithelial cells become cornified. After oestrus, the cornified cells disappear and large numbers of leucocytes appear.

In humans, the cycle which is referred to as menstrual cycle lasts about 28 days. The start of the cycle is taken to be the initial discharge of blood known as menstruation, and this event can easily be identified. The flow of blood, which lasts about five days, is due to the lining of the uterus being shed, along with a little blood. During the following days the lining regenerates in readiness for a fertilized ovum. By day 14 it has thickened considerably and the Graafian follicle releases its ovum into the oviduct, the process being called ovulation. (Tooles and Tooles, 1991). The menstrual cycle continues until menopause at the age of 45 - 50 years.

2.3.1.1 Histological Change in the Vaginal Epithelium during the Oestrous cycles

It has been observed that the histology of vaginal epithelium does not remain constant during the oestrous cycle. The vaginal epithelium is cyclically torn down and rebuilt, fluctuating between stratified - squamous and low cuboidal types. These cyclic changes can be followed by means of a vaginal smear technique in which the lumen is swabbed out and cells obtained examined under a microscope.

The oestrous cycle of the rat occurs usually every 4 or 5 days and each is characterised by periodic histological changes in the epithelium of the vagina and uterus. Asdell (1946) has described exfoliated cells associated with various stage of the oestrous cycle. He reported that as proestrus approaches, the cell types in the

vaginal fluids consist of nucleated epithelial cells and leucocytes with occasional cornified cells. The beginning of oestrus usually occurs when the smear contains about 75% nucleated and 25% cornified cells. Cornified cells are eventually the only ones present. Gradually, nucleated epithelial cells disappear. During the latter part of the cornified stage the vaginal fluid becomes abundant, and oestrus may end during this stage. The end of oestrus is followed by the appearance of large numbers of leucocytes and the virtual disappearance of cornified cells (Nalbandov, 1976).

2.3.1.2 Oestrogen and Changes in Vaginal epithelium

The rapid growth and cornification of vaginal epithelium during early and late oestrus have been found to be caused by oestrogen. When in the normal cycle the level of oestrogen drops after ovulation, or when in castrated female injection of oestrogen is stopped, the cornified vaginal epithelium begins to break down, the scales disappear and leucocytes become predominant (Nalbandov, 1976). Vaginal epithelial cycles and the hormone changes associated with these cycles have been characterized for many spontaneously ovulating rodents. In these rodents, the regular follicular cycles associated with spontaneous ovulation are reflected in predictable changes in plasma oestrogen and progesterone and consequently in changes in vaginal epithelium. Examination of a vaginal smear of a spontaneous ovulator therefore provides an indication of the hormonal state of the animal. For this reason, the terms used to describe vaginal smears for animals in different (pro-oestrous, oestrus, metoestrus, and dioestrus) phases of the cycle connote specific oestrogen and progesterone concentrations (Nalbandov, 1976). Thus in the vaginal smear technique, the type of cells predominating in the smear give a clue whether vaginal epithelium is or not being stimulated by oestrogen and progesterone (Nalbandov, 1976).

2.3.1.3 Hormonal Control of Oestrous Cycle

The recruitment of ovarian follicles, their maturation and eventual fate is controlled normally. The progressive growth of an ovarian follicle seems to be associated largely with changes in its receptor population, which in turn allows the follicle to respond to circulating hormones and develop new functional capabilities as maturation proceeds (Goldsworth *et al.*, 1981)

The control of the menstrual cycle (in human) is an excellent example of hormone interaction. The action of one hormone is used to stimulate or inhibit the production of another. There are four main hormones involved, two produced by the anterior lobe of the pituitary gland at the base of the brain, and two produced by the ovaries. The pituitary hormones (gonadotrophic hormones) stimulate the production of hormones from the ovaries. The functions of the gonadotrophic hormones (FSH and LH) and the ovarian hormones (oestrogen and progesterone) are as follows:

The Follicle Stimulating Hormone (FSH) causes Graafian follicles to develop in the ovary and also stimulate the ovary to produce oestrogen. The oestrogen causes repair of the uterine lining, which was broken down during the previous cycle, stimulates the pituitary to produce leutenizing hormone (LH). The Leutenizing hormone (LH) also stimulates the ovary to produce progesterone from the corpus luteum. The Progesterone causes the uterine lining to be maintained in readiness for the blastocyst (young embryo). The progesterone also inhibits the production of FSH by the pituitary.

The first hormone produced is FSH. FSH also stimulates the production of progesterone. Progesterone at the end of the sequence inhibits the production of FSH. The absence of progesterone now means that the inhibition of FSH ceases and so progesterone production commences again. In turn all the other hormones are

produced. This alternate switching on and off of the hormones produces a cycle of events - the menstrual cycle. Oestrogen and progesterone can also inhibit the production of FSH and LH respectively (Tooles and Tooles, 1991).

2.3.2 THE OVARIES (STRUCTURE AND COMPOSITION)

The female gonads, the paired ovaries, lie suspended in the abdominal cavity by the ovarian ligaments. The external coat is made up of germinal epithelium which begin to divide to form ova while the female is still a foetus. At birth (in humans) around 400,000 cell would have reached prophase of the first meiotic division and are called primary oocytes (Tooles and Tooles, 1991). Nearly all mammals are born with their full complement of oocytes. The oocytes remain in prophase for various periods according to the species (Baker *et al.*, 1977).

The oocytes remain in this condition until they are stimulated to resume meiosis and develop into mature cells called Graafian follicles through primary follicles. Each of the mature Graafian follicles contains a secondary oocyte or ovum. At ovulation, a mature Graafian follicle releases its ovum and the remains of the ruptured follicle form the corpus luteum. Follicles lie in a region of fibrous tissues in ovaries called the stroma. The stroma fills the rest of the ovary. The stroma provides the basic cellular matrix in which follicles are distributed.

The adult ovary is considered to be composed of three separate functional units, the stroma and interstitial tissue, the follicles, and the corpus luteum. These structures are in a converted state of flux and as one regresses another may be formed from its elements - the follicle is converted into the corpus luteum, and atretic follicles contribute to the stroma (Baird, 1984). In addition to the normally developing

follicles, an ovary always has a certain number of degenerating follicles and follicle undergoing atresia (Nalbandov, 1976).

It has been known for many years that the ovary secretes a variety of steroid hormones including oestradiol 17 - β and progesterone. More recently it has been discovered that the ovary also synthesizes a great variety of non-steroidal substances with hormonal action (Baird, 1984).

A single layer of surface epithelium covers each of the ovaries, which is continuous with the lining of the peritoneal cavity. The integrity of this covering is periodically broken at ovulation so that the surface epithelium is in a continuous state of damage, repairs and regeneration (Baird, 1984). The ovaries are well supplied with blood by an ovarian artery and numerous anastomosing branches from the uterine artery. Before entering the ovary the ovarian artery is often thrown into one or more loose coils (Harrison, 1962, Baird, 1984,). The venous drainage from the ovaries is by a network of venules, which surround the arteries. The close association of ovarian artery with uterine-ovarian vein into which the ovarian and uterine venous blood drain provides an anatomical basis for the transfer of hormones from vein to artery. This counter current transfer is a mechanism whereby products of the artery can feed back locally to influence ovarian functions. It is the means by which the uterus in many species induces regression of the corpus luteum. Accompanying the blood vessels is a rich net work of lymphatic and nerves (Baird, 1984).

3.3 OVUM DEVELOPMENT

As stated earlier the ovaries of adult mammals contain primary oocytes which develop into ova. The primary oocytes are formed from mitotic followed by meiotic divisions of the germinal epithelium (part of the external coat) of the ovary while the female is still a foetus. These divisions produce oogonia, which grow into the primary oocytes. The development of the primary oocytes into ovum involves meiotic divisions only.

The oocytes remain in prophase for various periods of time according to the species (Baker *et al.*, 1977), and then undergo meiosis as far as the diplotene stage. The oocytes remain in this condition until they are stimulated to resume meiosis within mature Graafian follicle and this period of delay can span many years.

The primordial follicles grow or develop into mature Graafian follicles, by enlargement and forming of coats. During the growth of the follicles they induce changes in the surrounding stroma cells. The primordial follicles which are flattened and single layered develop into a thick layer of cuboidal or columnar cells to form a distinct membrane granulosa. The layer becomes several cells thick. An outer encapsulating sheath derived from the stroma soon accompanies the enlargement of the granulosa layer. This constitutes the theca, later to be divided into a glandular, well vascularised inner layer, the theca interna and an outer one, or the theca externa composed of connective tissue (Harrison, 1962).

During the development of follicles, they enlarge as a result of proliferation of granulosa and thecal layers and in most mammals, one or more cavities soon form in the granulosa. The cavity enlarges, coalesce into an antrum and the fluid - filled follicle becomes surrounded by a uniform wall except at the point of attachment of the oocyte to the follicular wall. The stem of cells, which form this attachment, is called

cumulus oophorus and the cells that surround the oocyte form a layer called corona radiata. The mature follicle is now called the Graafian follicle (Harrison, 1962). Ovulatory follicles are those which have reached terminal stages of growth and maturation and have acquired the ability to ovulate following the ovulatory gonadotrophin surge or administration leutenizing hormone (LH) (Dubin and Wallance, 1988)

Each of the mature Graafian Follicles which are fluid - filled sacs, contains a secondary oocyte which is ovulated or released from the Graafian follicles as an ovum (Toole and Tooles, 1991).

2.3.3.1 Hormonal Influence on Ovum development

The early stages of follicle growth involve follicle-stimulating hormone (FSH). Primary follicles do not contain LH receptors, but their granulosa cells possess FSH receptors. FSH is responsible for recruiting follicles from ovarian 'pool' and stimulating their growth.

Maturation of the granulosa cells is promoted by FSH under whose influence they divide mitotically, proliferating around the lining of the follicle; their increased secretory activity is reflected in the growing volume of follicular fluid, in which the still quiescent oocyte is bathed (Goldworthy *et al.*, 1981).

Greatly accelerated growth occurs in the antral follicles, leading to much larger follicles called vesicular follicles. This accelerated growth is caused by the following mechanism. (Beato, 1988).

A. Oestrogen is secreted into a follicle and causes the granulosa cell to form increasing number of FSH receptors; this cause a positive feedback effect because it

makes the granulosa cells more sensitive than ever to FSH from the anterior pituitary gland.

B. The pituitary FSH and the oestrogen combine to promote luteinizing hormone (LH) receptors as well on the granulosa cells thus allowing LH stimulation of these cells in addition to the FSH stimulation and creating a very rapid increase in follicular secretion.

C. The increasing oestrogens from the follicle plus the increasing LH from anterior pituitary gland act together to cause proliferation of the follicular cells and to increase their secretions as well (Beato, 1988).

2.3.4 OVULATION

A Graafian follicle can reach a diameter of 1 cm (in humans) before it releases its ovum (Tooles and Tooles, 1991), a process called Ovulation. Ovulation is a critical point in the reproductive process of any female animal and the classification of mammalian species into either induced (reflex) or spontaneous ovulators has been a common practice for many years. A reflex ovulation is one that arises as a direct result of the acute initiation of a surge of LH secretion by external (sexual) stimuli. In practice the stimulation is normally provided by coitus and ovulation follows after a characteristic time interval. In contrast, spontaneous ovulations are primarily the result of an LH surge initiated by endogenous mechanisms involving endocrine feedback and is not dependent on any immediate specific external stimuli. Spontaneous ovulation tends to occur at regular interval in the unmated female and knowledge of the endocrine state of the female may allow a fairly accurate prediction of timing of the ovulation (Milligan, 1982).

The ovulatory follicle has some specific features as compared to the other large follicles present at the same time. These are as follows:

- a. A high oestrogens to androgens ratio in its follicular fluid. In contrast, the other large follicles, which are atretic, demonstrate a low ratio since atresia is associated with a loss of aromatizing ability by granulosa cells.
- b. The presence of functional LH receptors on granulosa cells. Such receptors are non-functional or absent in atretic follicles
- c. A high blood flow compared to that reaching atretic follicles.
- d. A high inhibin production (Dubin and Wallace, 1988)

Thinning of the follicular wall and the overlying surface epithelium, which produces a transparent area or stigma, precedes rupture. Although there is a significant increase in the volume of follicular pressure prior to rupture (Baker, 1984).

There is substantial evidence that the changes in the follicle preceding rupture are due to the release of collagenase enzymes. The granulosa cells are also known to produce another proteolytic enzyme, plasminogen activator (plasmin), the synthesis of which is stimulated by FSH (Baird, 1984). The importance of these two enzymes (collagenase and plasmin) has been demonstrated by the blockage of ovulation by a reduction of inhibitors of plasminogen activator, plasmin and collagenase activities (Brannstrom *et al.*, 1989).

The process is further advanced by the proteins released by this initial digestion, provoking an inflammatory response, with leucocytic infiltration and release of prostaglandins and histamine. These substances inhibit repolarization of the connective matrix and probably increase the local blood supply to the follicle (Baird, 1984). Just before ovulation, the basal lamina separating granulosa and theca cells migrate inside the follicle. Specific changes also occur at the apex level. Local

ischaemia induces death of the ovarian epithelium cells. Hydrolases produced by these dying cells' contribute to the digestion of the underlying cell layer. Hence, it is the disintegration of the follicular apex which results in follicular rupture and not increase in intrafollicular pressure or follicle contraction (Brannestrom *et al.*, 1989).

At the moment of ovulation the cumulus mass is extruded slowly together with rather viscous follicular fluid (Baird, 1984).

The corpus luteum is formed from the granulosa and thecal cells of the follicle ruptured at ovulation. The number of corpus lutea present in the ovaries is directly related to the eggs shed, and varies with the species. In monovular species such as human, ovulation occurs at random in either ovary (Baird, 1984). After ovulation the follicular cavity is filled with blood and lymph. In some species, e.g. swine, the fluids distend the ovulated follicle so much so that for five to seven days after rupture it is larger than at any time before. In other species such as in cattle and sheep, the accumulation of fluids is insignificant, and the follicle is smaller than it was before ovulation (Nalbandov, 1976). The newly formed corpus luteum rapidly becomes invaded by blood vessels, and these follow strands of thecal cells (Baird, 1984).

The increase in weight of the corpus luteum occurs because of hypertrophy and hyperplasia (Nelbandov, 1979) but largely due to the former (Baird, 1984).

2.3.4.1 Hormones and ovulation

The final crucial maturational effect of FSH, which is potentiated by oestrogen, is the induction of reception for LH on the granulosa cells. These permit the ovulatory actions of LH to be exerted on preovulatory follicles after the LH surge arrives; there is little further involvement of FSH in the ovulatory mechanism (Goldwerthy *et al.*, 1981).

The LH receptors are exclusively located in the thecal layer, which at this stage has developed around the basement membrane of the follicle. The cells can synthesize steroids, and in response to LH produce androgens. LH causes the granulosa cells to luteinize i.e. the cells capable of producing progesterone in large amounts (Goldsworthy *et al.*, 1981). The gonadotropin surge *in vivo* or the addition of gonadotropins to follicles kept *in vitro* simulates progesterone secretion whilst high oestradiol to progesterone ratio is often preceded by a transient and overall increase of steroid secretion. These changes play a role in the oocyte's cytoplasmic maturation. *In vivo*, the increase in progesterone is essential for ovulation although this is not obvious *in vitro* (Janson and Brannstrom, 1989).

2.3.5 THE UTERUS (WOMB)

The uterus or womb is a thick walled, pear shaped hollow muscular receptacle in which the fertilized ovum develops throughout the embryonic and the foetal stages until birth. The human uterus is 7.5 – 9 cm long, 5 – 6 cm wide in its widest part and 2.5 -3.5 cm thick in its thickest part. It is flattened in front and convex behind. The tapering end of the uterus is the cervix, which projects into the upper vagina. The cavity of the uterus is triangular in shape when seen from the front (Anto, 1991).

The uterus (body) has three coats namely serous, muscular and mucous. The muscle coat is known as the myometrium. The mucous lining of the uterus is the endometrium. It is thick and lined with columnar cells many of which are ciliated. It also possesses numerous tubular glands, which extend to the muscle coat. The part of the body situated above the level of the insertion of the fallopian tube is the fundus. The area of insertion of the fallopian tube into the uterus is the cornu. The tapering before the cervix is the isthmus. It is important because it is believed that it becomes the lower uterine segment during pregnancy and labour (Anto, 1991).

2.3.6 FERTILIZATION

The fertilization of ovulated eggs obviously depends on the meeting of germ cells in the oviduct at a time when sperm and eggs are able to fertilize and can be fertilized (Nalbandov, 1976). Once the sperm has entered the ovum, the nucleus of the ovum discharges the second polar body, allowing the 23 unpaired chromosomes of the female pronucleus and 23 unpaired chromosomes of the male pronucleus to align themselves to reform a complete complement of 46 chromosomes (23 pairs) in the fertilized ovum (Jones, 1986). If the timing is proper and adequate quantities of viable sperms are present, the fertilization rates of all polytoccous animals approach

100 percent. In rabbits, guinea pigs, rats, and mice the rates rarely drop below 95 percent according to most studies; the average of these species is 98.6 percent (Nalbandov, 1976).

2.3.7 IMPLANTATION AND UTERINE RECEPTIVITY

After fertilization and the early cleavage of the egg which are completed in the oviduct, the young embryo consisting of from 8 to 16 cells (blastocyst stage), arrives in the uterus. During the first day after its arrival in the uterus, the embryo is completely dependent upon uterine secretions for its energy until it implants itself and forms a permanent placental connection with the maternal circulatory system (Nalbandov, 1976).

Implantation of ova in rodents involves a series of morphological and biochemical interaction between the blastocyst and the uterus culminating in the attachment of the embryo to the mother by means of placenta (Cook and Hunter, 1978). During these interactions, the uterus undergoes complex alterations before and after implantation in many species including the human. These are collectively termed decidualization. One of the earliest signs of blastocyst implantation in all species investigated is a localised increase in endometrial vascular permeability (Psychoyos, 1973). Psychoyos, in 1961, showed originally in rats that the blood vessels near site of an implanting blastocyst become more permeable, so that after the injection of a large molecular weight coloured dye, such as Puntamine Sky Blue, a coloured band appears across the uterus where a blastocyst is present. Wilson (1963) demonstrated the passage of cells, which he called primary invasive cells, from the blastocyst into the uterine epithelium. Finn and Hinchliffe (1964) showed that the

enzyme alkaline phosphates appeared in the endometrial stroma around an implanting blaslocyst.

The control of implantation operates at two levels, systemic and local. A necessary uterine endometrium should be appropriately prepared. The endometrium is under the control of steroid hormones; in some species at least the correct sequences and concentration of progesterone and oestrogen is crucial if implantation is to occur successfully (McClaren, 1985). Implantation is dependent on a basic hormonal sequence, which is common to all species. This includes :

(A) A preovulatory surge of oestrogens secreted by the growing follicles, which have a preparatory effect on the endometrial maturation.

(B) A luteal secretion of progesterone, and in some cases of oestrogens, at the time of implantation.

The primordial role of ovarian oestrogens in implantation has been clearly established in myomorph rodents (Rats and mice). In human endometrium, the preovulatory surge of oestrogens induces a non-synchronous synthesis of oestradiol and progesterone receptors during the midluteal phase of the cycle.

If the secretion of this implantation oestrogen is suppressed as occurs during lactation or through experimented interference, the blastocysts fail to implant and enter into a condition of dormancy characteristic of delay implantation (McClaren, 1985).

At the local level the presence of a viable blastocyst is a minimal requirement. Interactions between the embryo and the adjacent endometrium may be critical, but they are not easily emendable to study at the cellular or biochemical level, even in laboratory animals (McClaren, 1985).

2.3.8 EMBRYONIC SUPPLY IN UTERUS

The initial stage of implantation involves the interaction between the trophoblast cell of the blastocyst and the epithelial cells lining the uterine lumen. The trophoblastic wall of the blastocyst could be regarded as a membrane, the main function of which is to supply nutritive material for the immediate needs of the egg. No part of the trophoblastic wall becomes incorporated in the definitive embryo. It is an extra embryonic ectodermal layer (Boyd and Hamilton, 1952). Chorionic villi develop an enormous surface of exchange. Terminal branches of spiral arteries, which enter the intervillous space, convey maternal blood.

Oxygenation of foetal blood and elimination of carbon dioxide take place in placental villi. Oxygen dissolved in maternal blood passes directly into the foetal blood. In contrast, carbon dioxide is higher in foetal blood and diffuses towards the maternal blood which return to the placenta via the umbilical arteries (Jennet, 1989).

2.3.9 OVA WASTAGE

The fertilization rate of litter bearers approaches 100 percent but the implantation rate and the number of young carried to term, in the great majority, are significantly less than the number of ova fertilized. The discrepancy between the number of ova fertilized and the number of embryos found in the uterus varies greatly between the individuals of one species but it is nearly constant for polytocous species. Several studies of rats, rabbits, pigs and other litter bearers have shown that 30 - 50 percent of the fertilised eggs are lost sometime during gestation. Most of the foetal mortality occurs during the first third of pregnancy, and probably most of the losses occur between fertilization and implantation (Nalbandov, 1976). Osei Bonsu (1988) showed that ova prenatal lose in the albino rat was between 6 - 67 percent.

Substantial prenatal mortality has been observed in all animals studies, although there are differences between species the extent and timing of the death. (Wilmot *et al.*, 1986).

Broadly speaking all pregnancy losses and birth defects are the result either of genetic abnormality in the foetus or child or an adverse environment. The latter can be the result of suboptimal conditions for foetal growth and development as a consequence of inadequacy or abnormality of the mother's reproductive system, or the result of exposure of the foetus to an environmental insult, such as an infectious agent or a harmful drug (Jacobs, 1982).

Every organism has a specific number of chromosomes and these chromosomes contain characteristic array of genes, which control all processes of growth and development. Any gain or loss of chromosomal material almost invariably results in gross abnormalities of the foetuses, which prejudices its survival. Gain or loss of chromosomal material occurs as either a numerical abnormality, that is, the presence or absence of one or more chromosomes or as in size or shape of one or more chromosomes (Jacobs, 1982). Gross chromosomal abnormalities are present in 50 - 61.5 percent of spontaneous human abortuses of the first and second trimesters (Boue *et al.*, 1975).

According to Wilmot and others (1986) embryos may be abnormal because of errors at meiosis. Structural abnormalities are the result of chromosomes breaking and rejoining in an abnormal way. Various chemicals and X-ray have been shown to produce structural abnormalities of the chromosomes in the germ cells of laboratory animals.

In humans, production of chromosomal abnormal gametes have been strongly associated with advancing maternal age (Jacobs, 1982). Adam (1970) found that in

the rabbit, the incidence of "arrested cleavage" was higher in old does than in the young ones. He suggested that the defects reside in the egg.

A number of mutations affecting prenatal development have been described for mice. Embryos homozygous for the yellow allele of the agouti locus die in the late blastocyst stage, though they do provoke a decidual reaction (Pederson, 1974)

The maternal environment may be inadequate for the support of a normal pregnancy either because the reproductive tract is inherently abnormal or as a result of an inappropriate hormone pattern (Wilmot *et al.*, 1986)

There is a reduced decidual response in aged mice (Finn, 1960) and bank voles (Nerquaye-Tetteh and Clarke, 1987). It has been suggested that this reflects difference in vascular supply and collagen content. Embryos may die because they are not at the correct stage of development for the particular uterine environment. Loss may result from an inappropriate distribution of embryos within the uterus (Wilmot *et al.*, 1986).

Although the placenta theoretically prevents the passage of serum from the mother to the foetus, some pathogenic microorganisms can contaminate the foetus; syphilis, toxoplasmosis and paludism, viruses easily cross the placental barrier and can be responsible for very serious embryonic and foetal disorders German measles, herpes, hepatitis, HIV (Buelke -Sam and Holson, 1980).

2.3.10 ABORTIFACIENTS

Certain drugs that are used in cancer chemotherapy and as immuno suppressants are embryotoxic and some have been used as abortifacients.

Quinine, an anti-malarial drug has a reputation as an abortifacient. Used in combination with ergot, it causes contraction of uterine muscles, especially once

labour has begun. More recently it has been used for the expulsion of uterine contents following intrauterine death of the foetus (Bowman and Rand, 1980). In the 17th century, nutmeg, which was used in Europe mainly as a medicine, was also used as an abortifacient (Bowman and Rand, 1980). Drugs that enhance uterine motility are termed oxytocics after the hormone oxytocin (the prototype). These oxytocics stimulate uterine contraction and thereby hasten labour either at term or prematurely.

Compounds exhibiting pronounced oxytocic activities are quite varied in their chemical structure. The naturally occurring compounds include hormones of the posterior pituitary namely oxytocin and vasopressin, the ergot alkaloids of the ergonovine group and the prostaglandin which are the latest groups of oxytocics to be studied. Synthetic substances comprise modified analogues of oxytocin and ergonovine, model compounds based on the structure of ergot alkaloids, certain derivatives of quinolizidine and some aminoethyl derivatives of phenols. Besides these a number of botanical drugs and synthetic substances have been found to possess oxytocic activity to various degrees but are not of medicinal interest (Burger, 1970).

The work of Goonasekera and others in 1995 indicated the abortifacient properties of the fruits of *Jatropha curcas*. The extract used interrupted pregnancy at early stage after implantation.

CHAPTER THREE

MATERIAL AND METHODS

3.1 COLLECTION AND EXTRACTION OF PLANT MATERIAL

3.1.1 Collection of Fruits (Including the Seeds) of *Jatropha curcas*

After establishing the taxonomic identity (see 2.1.1), fruits of *Jatropha curcas* including the seeds were collected from two places around the campus of the Kwame Nkrumah University of Science and Technology Kumasi. The places were Kotei and Atonsu located south and west of the University campus respectively. The leaves and fruits of *Jatropha curcas* are shown on plate 3.1

The fruit, which included both dry and fresh ones, were air-dried for three weeks.

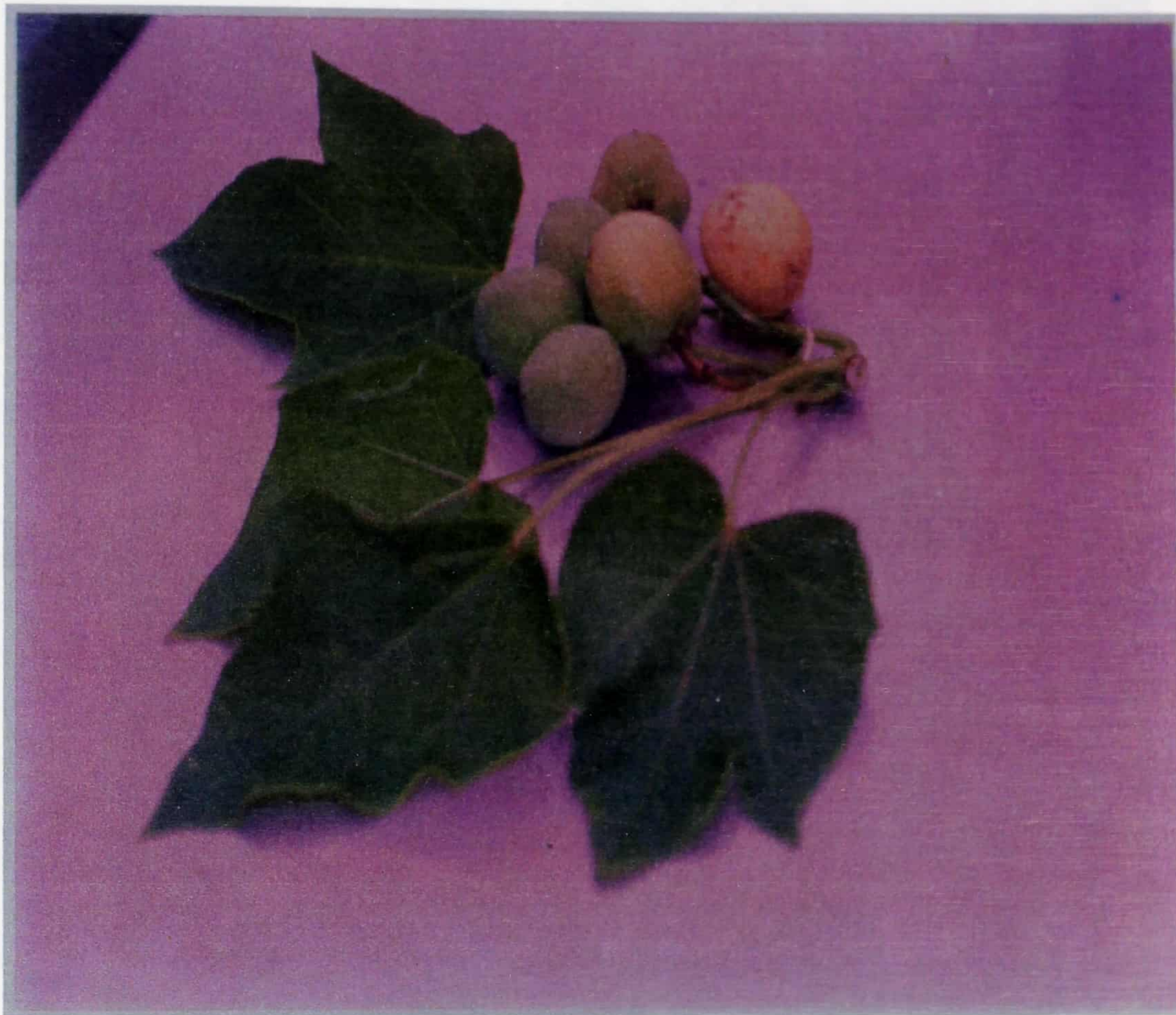


Plate 1 Leaves and fruits (fresh) of *Jatropha curcas*

3.1.2 Preparation of Alcoholic Extract of *Jatropha curcas*

Two kilograms of the dry fruit (including seeds) of *Jatropha curcas* was ground into powder and subjected to Soxhlet extraction using 70% ethanol as a solvent (Fig 3.2). The solution obtained from the Soxhlet extraction apparatus was put in a rotary evaporator to reduce it to a syrup. The syrup was then put into a vacuum evaporator to evaporate it to a constant weight. The extract was stored in a refrigerator and different dosages made for the administration when needed.

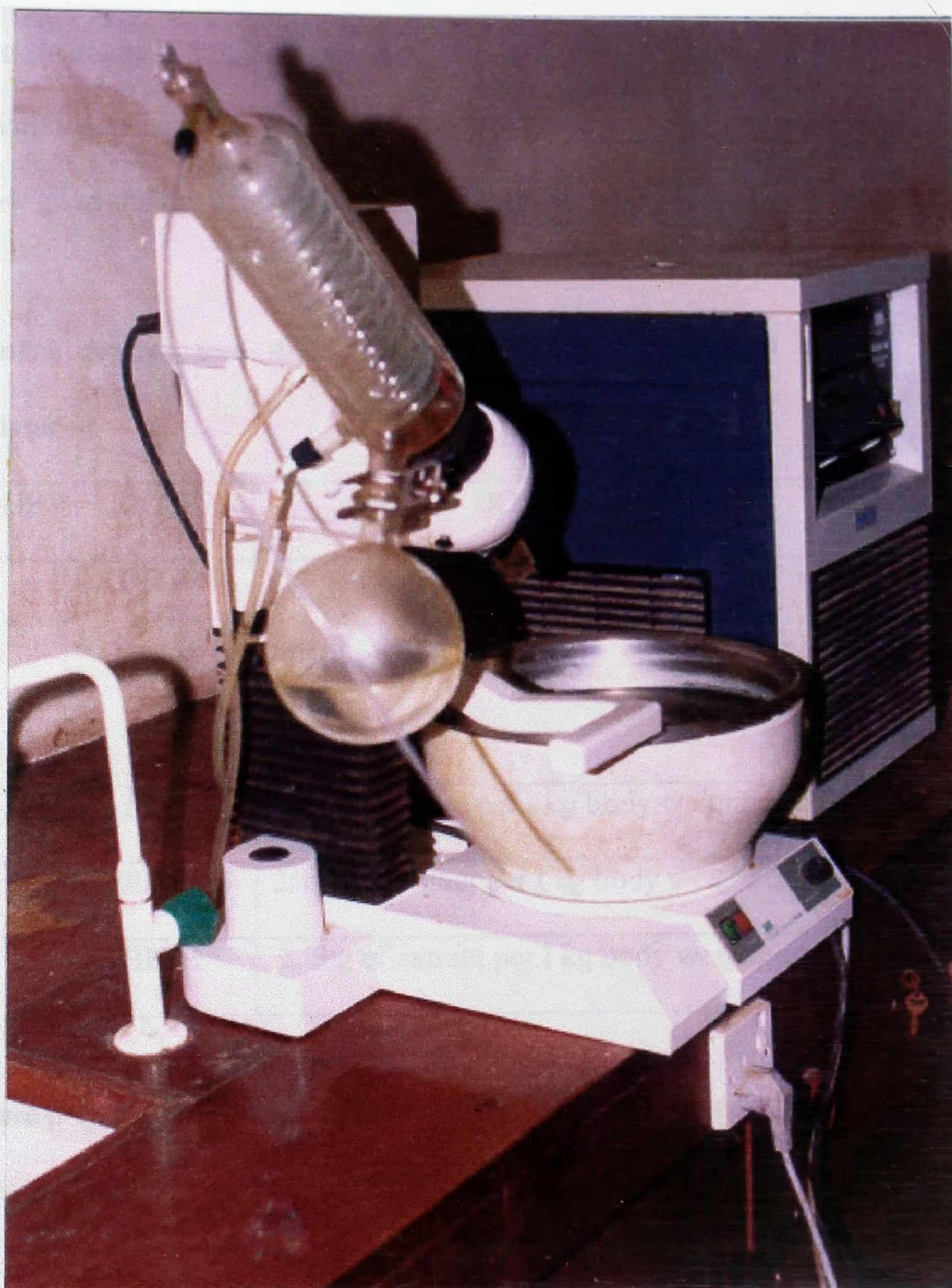


Plate 2 Soxhlet Apparatus (used for the extraction)

3.2 DOSE LEVELS AND PREPARATION OF SOLUTIONS

After the LD₅₀, (the dose at which 50% mortality occurred in the population of rats used was establish to be 12.5 g/kg, the highest dose level of 2.5g/kg (1/5 of LD₅₀ was chosen for the experiment. Lower dosages of 2.0 and 1.5 were also chosen to appreciate the graded effect of the extract if any. The control dose contained no amount of the extract (0g/kg).

The extract at state doses of 2.5, 2.0, 1.5 and 0g / kg body weight of rat were orally administrated using distil water as the vehicle.

The table below gives the dose levels administrated and their designated dose numbers.

Table 3.1 Treatment groups and dose levels of *Jatropha curcas* administered

TREATMENT GROUP	DOSE	DOSE LEVEL
T ₀	CONTROL	1.0 g of extract per I kg body weight of rat (distil water) (control)
T ₁	LOW	1.5 g of extract per I kg body weight or rat
T ₂	MEDIUM	2. 0 g of extract per I kg body weight of rat
T ₃	HIGH	2. 5 g of extract per I kg body weight of rat

The solutions used were prepared freshly every day by dissolving the required quantity of the extract in 1 ml of distil water.

3.3 ADMINISTRATION OF DOSAGES

A 16 – G blunted needle cannula was used. The dams were held firmly by the skin at their backs and the needle, attached to a syringe containing the required dosage in 1ml of water solution was negotiated gently into the back of the throat. It was then gently pushed downward through the oesophagus and the solution was then gently released into the gut of the rat.

3.4 TEST ANIMALS

3.4.1 Collection and Maintenance of Female rats

One hundred and twenty (120) female albino rats were collected from the Animal House of the Biological Science Department of the Kwame Nkrumah University of Science and Technology, Kumasi. These animals were strictly in-bred, and kept under a uniform environmental conditions. They are genetically identical strains. The albino rats were housed singly in wooden cages, which measured 30 cm x 25 cm x 17 cm. The rats were allowed to acclimatize in the cages for three (3) weeks at room temperature. Each cage had a wood bedding of shavings on their floors which was changed once every week.

Each afternoon, the rats were fed with water and feed ration consisting of 50% maize (milled corn), 30% concentrates and 20% wheat bran. The concentrates contained the following food substances in percentages.

Protein	-	15.79	Methionine	-	0.32
Oil	-	4.36	Calcium	-	3.10
Fibre	-	5.20	Phosphorus	-	0.62
Lysine	-	0.79	A.V. Phosphorus	-	1193.25

(Source: VAKS FARM, feed mills P O Box 3119, Kumasi)

The feed was put in food hoppers and drinking bowls were used to serve the animals with water.

4.2 Vaginal Smear, oestrous stages and Selection of rats for the experiments

To establish the cyclicity of the female rats. A platinum loop was moistened with water and used to scrape the walls of the vagina while the animals were held in a tight position. The loop was rinsed in a drop of physiological saline (0.9 g NaCl in 100 ml of distilled water) on a slide. The slide was then observed under the microscope.

The female animals whose vaginal smears revealed the four successive stages of the cycle for two consecutive times were considered as animals with regular oestrous cycles, and it was these, which were selected for the experiment from the large stock. The stages were

- (i) Proestrus (nucleated epithelial cells and leucocytes with occasional cornified cells in smear)
- (ii) Oestrus (abundant cornified cells in smear)
- (iii) Metoestrus (abundant leucocytes in smear)
- (iv) Dioestrous (absence of leucocytes in smear)

In all, ninety-six (96) female rats with regular cycles were selected from the large stock for the work. These were between 100 and 150 days old and were weighed between 150 g and 250 g.

The male rats selected for the experiments were those who looked physically fit and active and had been proved fertile (they have impregnated some female rats before).

3.4.3 Grouping and Identification of the Test Animals

The selected female rats were randomly divided into four (4) groups using the randomization tables. The groups were given the different dose levels of the extract

Labels placed on its cage identified every female rat in each of the four treatment groups.

3.4.4 The use of the grouped animals

The first eight-(8) female rats in each group were used for the experiment to determine the effect of the extract of *Jatropha curcas* on oestrous cycle length development of ova and ovulation in the rat.

A second set of 32 female animals similarly grouped into batches of eight (8) animals in each treatment group were used to determine the effect of the extract on conception or pregnancy and implantation.

A third set of 32 animals was used for the determination of the effect of the extract on ova wastage (abortion).

The observed changes in weights of the animals were also recorded during some of the work.

3.4.5 Mating Procedure and Pregnancy

When there was the need for mating and establishment of pregnancy in the experiments, the female rats that showed proestrous smears were paired with males in the evening. They were examined the following day for positive mating (pregnancy which) was indicated by the presence of sperm in the vaginal smear of the female rats, or and the

presence of copulatory plug in the vaginal orifice. The day of positive mating was considered day zero (O) of pregnancy.

Each of the female rats was weighed once on every 4th day. The results were recorded and they were used to determine the increase or decrease in weight with the administration of the in weight with the administration of the extract.

3.5 HISTOLOGICAL EXAMINATIONS

The ovaries and uterus of some of the animal were removed and prepared for histological sections. The sections were mounted on the microscope for examination.

3.5.1 Preparation of Section of the Ovaries and Uteri

The removed ovaries and the uteri were kept in Bouin's solution for 24 hours in order to fix them. They were transferred into 70% alcohol and left overnight. Passing them through 30%, 50% 70%, 90% and absolute alcohol then dehydrated the tissues. The ovaries and uteri were then put in a mixture of absolute alcohol and xylene (50,05) for 30 minutes and further cleared in xylene until they looked transparent. This ensured complete removal of alcohol from the tissues. They were then impregnated with wax by passing them through three changes of molten wax. They stayed for one hour in the molten wax for each change. After the wax had solidified, they were trimmed and the fixed ovaries and uteri were sectioned. Using the microtome. The sections were stained mounted and photomicrographs were taken from them for observation.

3.5.2 Staining and mounting of section of ovaries and uteri

The cut sections were placed on hot plates at about 50°C to allow them to flatten. They were then mounted on clean glass slides and flooded with dilute albumin solution. They were stained in Enlich's Hematoxylin for 10 – 20 minutes and rinsed in distilled water after they were dewaxed in xylene and also dehydrated in a bath of absolute alcohol for 2 minutes and then in a bath of 90% alcohol for 1 minute. The stained slides were left under running tap until the pink sections turned blue ("blueing"). Overstaining and under staining were checked and corrected. They were then counter stained in Eosin for 2 minutes after the slides had been washed in 70% alcohol for 2 minutes. The counter stained slides were dipped in through 70% 90% and absolute alcohol and finally in xylene for 5 minutes in each change.

The slides were mounted on light microscopes and photomicrograph of the parts were taken and studied for possible characteristic changes as a result of the effects of the extracts on cells and tissues of the ovaries and uteri.

3.6 EFFECTS OF THE ALCOHOLIC EXTRACT OF THE FRUITS OF *Jatropha curcas* ON LENGTH OF THE OESTROUS CYCLE, DEVELOPMENT OF OVA AND OVULATION RATE IN THE ALBINO RAT

To determine the length of the estrous cycle of the animals, vaginal smear were made and examined each day after the administration of the extract. The day when relatively high numbers of cornified cells was observed in the vagina smear (oestrous stage) was taken as day 0 of the oestrous cycle, or the start of one oestrous cycle and for that matter the last day of proceeding oestrous cycle. The time lapse between two

consecutive oestrous stages was taken as the lengths of the oestrous cycle of the rat. The lengths of the estrous cycles of the animals in the different treatment groups including the control group were compared, statistically, to verify the effect of the alcoholic extract of *Jatropha curcas* on the lengths of the oestrous cycle.

Photomicrographs of the vaginal smear of some animals were taken for exhibition.

To determine the effect of the extract on the development of ova, the vaginal smear pattern was examined for the animals. Normal vaginal smear pattern indicates the production of hormones (oestrogen and progesterone) and for that matter normal development of ova.

The animals were scarified at the end of the administration period (16 days). The ovaries were removed and histological sections made from them, after the number of corpora lutea in them were counted and recorded.

The histological sections of the ovaries in different treatment groups were compared to determine the various stages of development of the ova in the different treatment groups. This provided additional information (in addition to the vaginal smear) on the effect of to extract on ova development.

Photomicrographs of sections the ovaries of some animals were taken for exhibition.

To determine the ovulation rate, the number of corpora lutea (pinkish yellow points in ovaries) were counted and recorded. The number of corpora lutea represents the number of eggs (ova) ovulated – ovulation rate.

3.7 EFFECTS OF THE ALCOHOLIC EXTRACT OF THE FRUITS OF *Jatropha curcas* ON POSITIVE MATING (PREGNANCY) AND IMPLANTATION IN THE ALBINO RAT

The animals, which were singly housed, received their required dosage of the extract for five (5) days before they were paired with healthy males, which had been proved fertile. The females were examined for the presence of sperms in the vaginal smear (positive mating). The number of female rats that showed positive mating (became pregnant) as compared to the number that did not show positive mating (did not become pregnant) was recorded. The means were compared. This gave an indication of effect of the extract on positive mating (considered as pregnancy in the rat).

The animals that showed positive mating were used to determine the effect of the extract on implantation. The administration of the extract continued for the pregnant animals until 5th day after pregnancy. By this time, the implantation process was assumed to have ended. Implantation in the rat occurs between the 4th and 5th day of pregnancy. On day 6 of pregnancy, the female animals were anaesthetized, through intra-peritoneal injection, using 60mg sagatal per 1 kg body weight of rat. Then, 0.2 5ml of 1% solution of Pontamine Sky Blue 5 BX dye was injected into the jugular vein of animals.

Fifteen (15) minutes after the injection of the Pontanmine Sky Blue 5 BX dye, the animals were dissected and the uterine horns exposed. Coloured (blue) bands appear across the places in the uterus where blastocysts were present and this enabled the implantation sites to be identified more easily. The number of the blue bands in the uterine horns gave the number of the implantation sites. The number of the implantation

sites were counted and recorded and the means for the different treatment groups including the control were compared.

Pictures of some dissected animals showing implantation sites were taken for exhibition.

Histological sections were made from the uteri of selected animals each of the treatment groups. The sections of the uterus of each of the four treatment groups were examined under the microscope and compared for any characteristic changes in the endometrial tissues (layers). Photomicrographs of the histological sections of the uteri of some animals were taken for exhibition.

3.8 EFFECT OF THE ALCOHOLIC EXTRACT OF THE FRUITS OF *Jatropha curcas* ON OVA WASTAGE OR FOETAL RESORPTION (ABORTION) IN FEMALE ALBINO RAT.

The third 8 animals in each of the four treatment groups were used for this experiment. These thirty-two (32) female rats, selected and acclimated weighed between 150 and 250g. They were individually housed and later mated. The day of positive mating (see 3.4.5) was considered day 0 of pregnancy. The rats used for this study were given the required dosages of the extract starting from day 6 pregnancy. By this time the developing embryos had implanted themselves to the walls of the uterus. The animals were dosed for 10 consecutive days and were sacrificed on day 17 of pregnancy. After the 17th day of pregnancy, the developing fetuses may not respond to the effect of the extract. Their ovaries were examined and the number of corpora lutea were counted and

recorded. The number of normal fetuses removed were also counted and recorded for each of the animals.

The differences between the number of fetuses removed and the number of corpora lutea gave the indication of the number of ova wastage or foetal loss or aborted. The comparison of means number of ova wasted in the different treatment control, gave an indication of the effect of the extract on include ova wastage or abortion.

3.9 EFFECT OF THE ALCOHOLIC EXTRACT OF FRUITS OF *Jatropha curcas* ON WEIGHT OF THE FEMALE RATS

During the administration of the extract, the weights of the female rats were taken once in every four-(4) days using the Mettler P 1200 balance.

The weights were also statistically analysed and their means compared to verify the effect of the extract on weights.

3.10 TOXICOLOGICAL EFFECT OF THE ALCOHOLIC EXTRACT OF FRUITS OF *Jatropha curcas* ON THE FEMALE RATS

The general health of the female animals was also observed during the experimental period. The number of deaths that occurred in the different treatment groups was recorded.

3.11 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The treatments were arranged in a Completely Randomized Design with 8 replications.

Analysis of variance was performed to determine statistical differences among the treatments. Significant differences between any two means was determined using the Least Significant Difference (LSD) approach at 5% level of significance.

CHAPTER FOUR

RESULTS

4.1 EFFECTS OF THE ALCOHOLIC EXTRACT OF FRUITS OF *Jatropha curcas* ON THE LENGTH OF OESTROUS CYCLE OF THE ALBINO RAT.

The mean lengths of the oestrous cycles of the rats are on the different doses of the extract of *Jatropha curcas* are shown in table 4.1 below.

Table 4.1

The Mean length of oestrous cycle of albino rats on different doses of the extract

Treatment group Dose Number	Dose Level (g/kg)	Mean Length of Oestrous Cycle (Days)
T ₀	0 (control)	4.40 ^a
T ₁	1.5	4.43 ^a
T ₂	2.0	4.37 ^a
T ₃	2.5	4.17 ^a

Differences in means not significant when superscripts are the same.

There were no significant differences among the means of the length of oestrous cycle of the animals of the different treatment groups.

4.2 EFFECT OF THE EXTRACT OF *Jatropha curcas* ON THE DEVELOPMENT OF THE OVA IN THE ALBINO RAT.

4.2.1 Vaginal Smears Patterns

Microscopic examination of the vaginal smears showed that the animals in each of the treatment groups showed normal pattern of vaginal smears irrespective of the dose

level of extract given to the animals. There was the normal pattern of production of vaginal epithelial cells in the smears of animals in each of the treatment groups. In fact there were no significant differences between the vaginal smear pattern of the control animals and those that were given the extract. Also, there were no significant differences among the vaginal smear patterns of the low, medium and the high dosed animals.

The photomicrographs, (Plates 3 to 10) compares the pattern of vaginal smear for a selected high-dosed animal and a selected control animal.

At early pro-oestrous stage, the vaginal smear observed contained some leucocytes (L) cornified cells and few nucleated cells. At late proestrous stage however, the smear were abounding in nucleated cells (N).

At the oestrous stage the abundant cells seen were the cornified cells (C).

The metoestrous stage of the smears, which lasted for a brief period, was difficult to be distinguished. This stage comes in between the oestrous and the Dioestrous stage.

At the dioestrous stage, the vaginal smears observed contained abundant leucocytes (L). From this stage, the early pro-oestrous stage was entered into again.

The above normal vaginal smear patterns was observed for all the animals in each of the treatment groups. The results show that the extract of *Jatropha curcas* used, did not affect the normal production of hormones for the follicular development and for that matter the extract did not affect the development of ova.

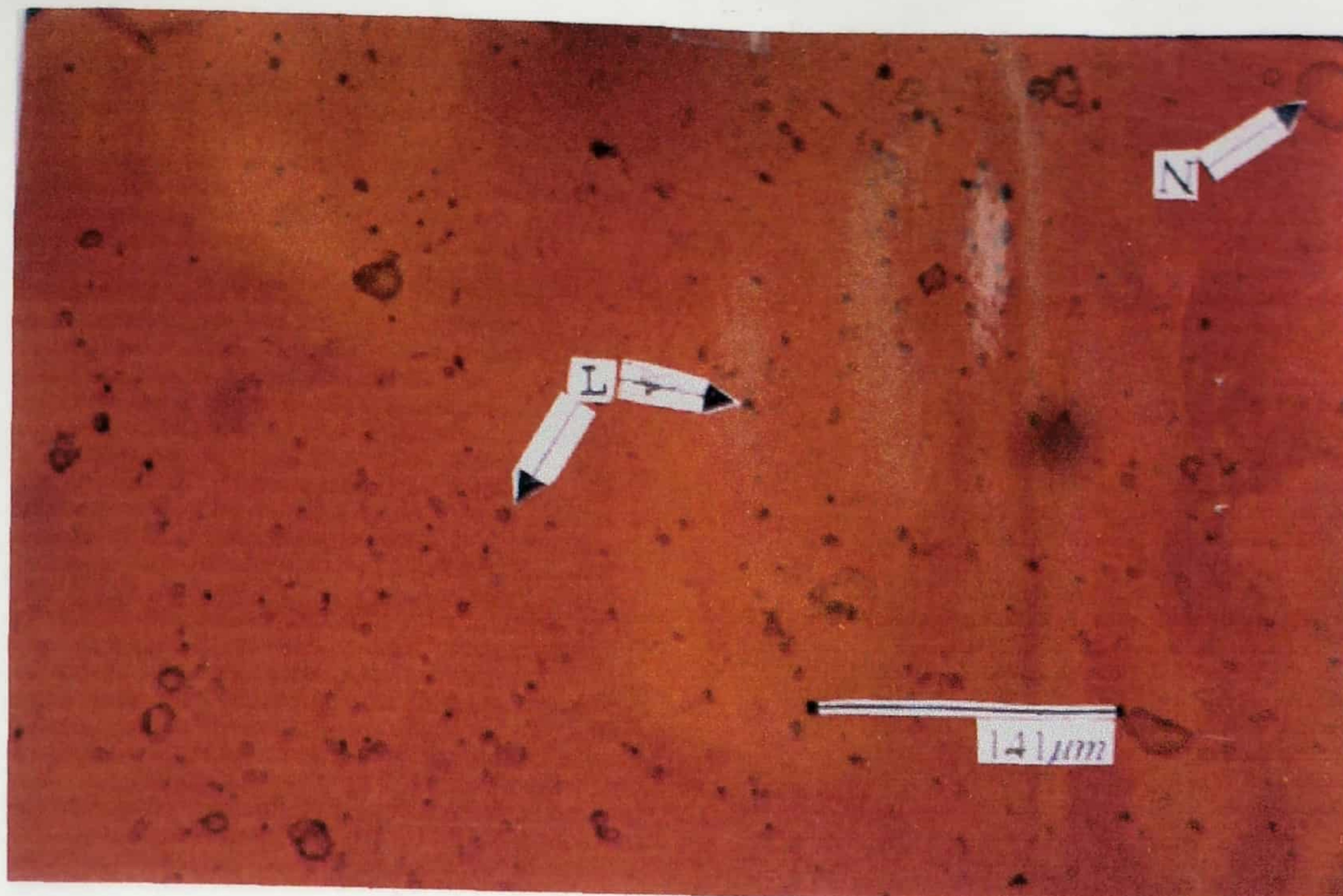


Plate 3

Photomicrograph of the vaginal smear of a **control** animal (x128), at the early **Protoestrus** stage. Note the presence of leucocytes (L) and few nucleated cells (N).

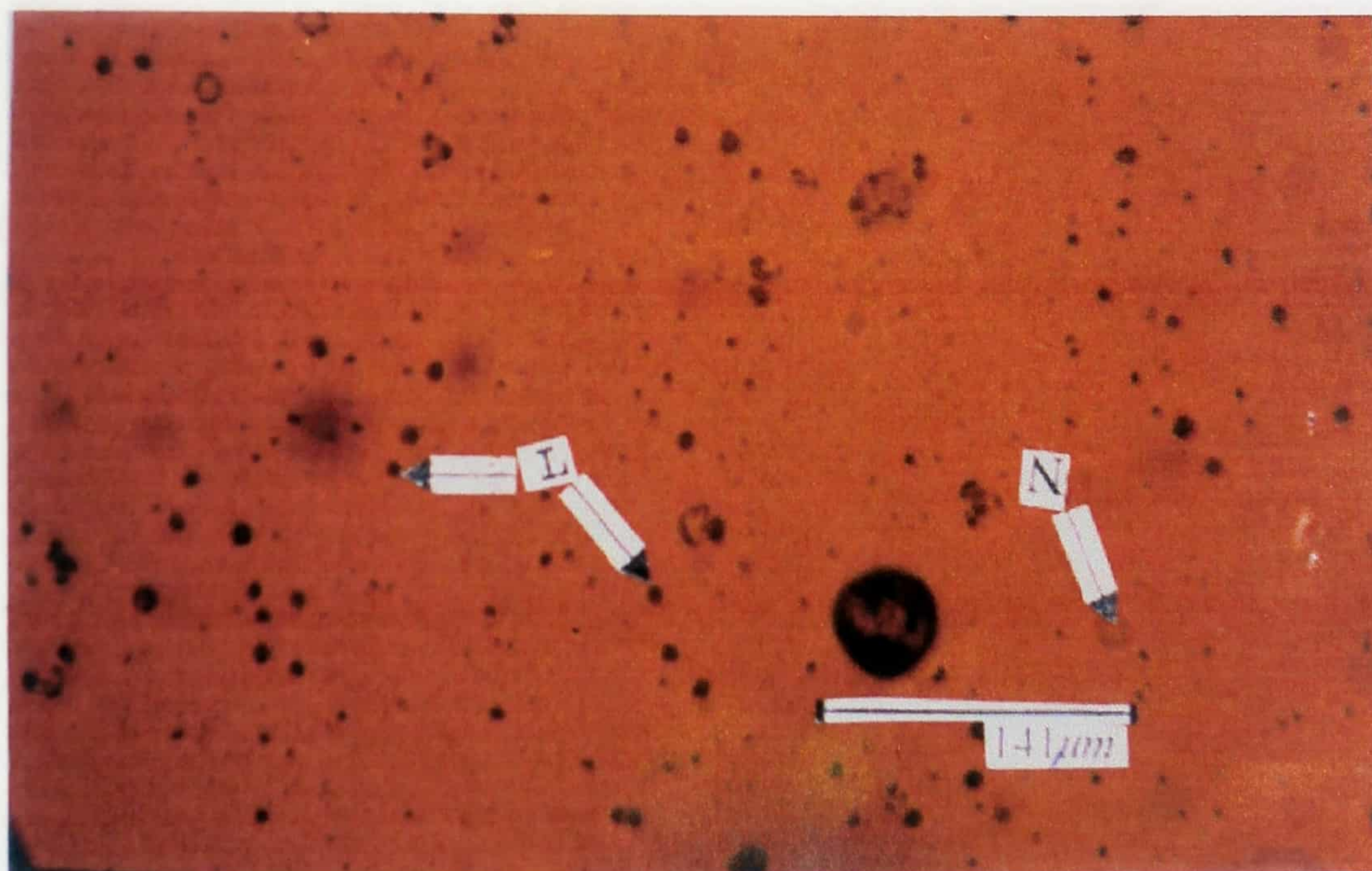


Plate 4.

Photomicrograph of the vaginal smear of a **high dosed** animal (x128), at the **early Proestrus** stage. Note the presence of leucocytes (L) and few nucleated cells (N).

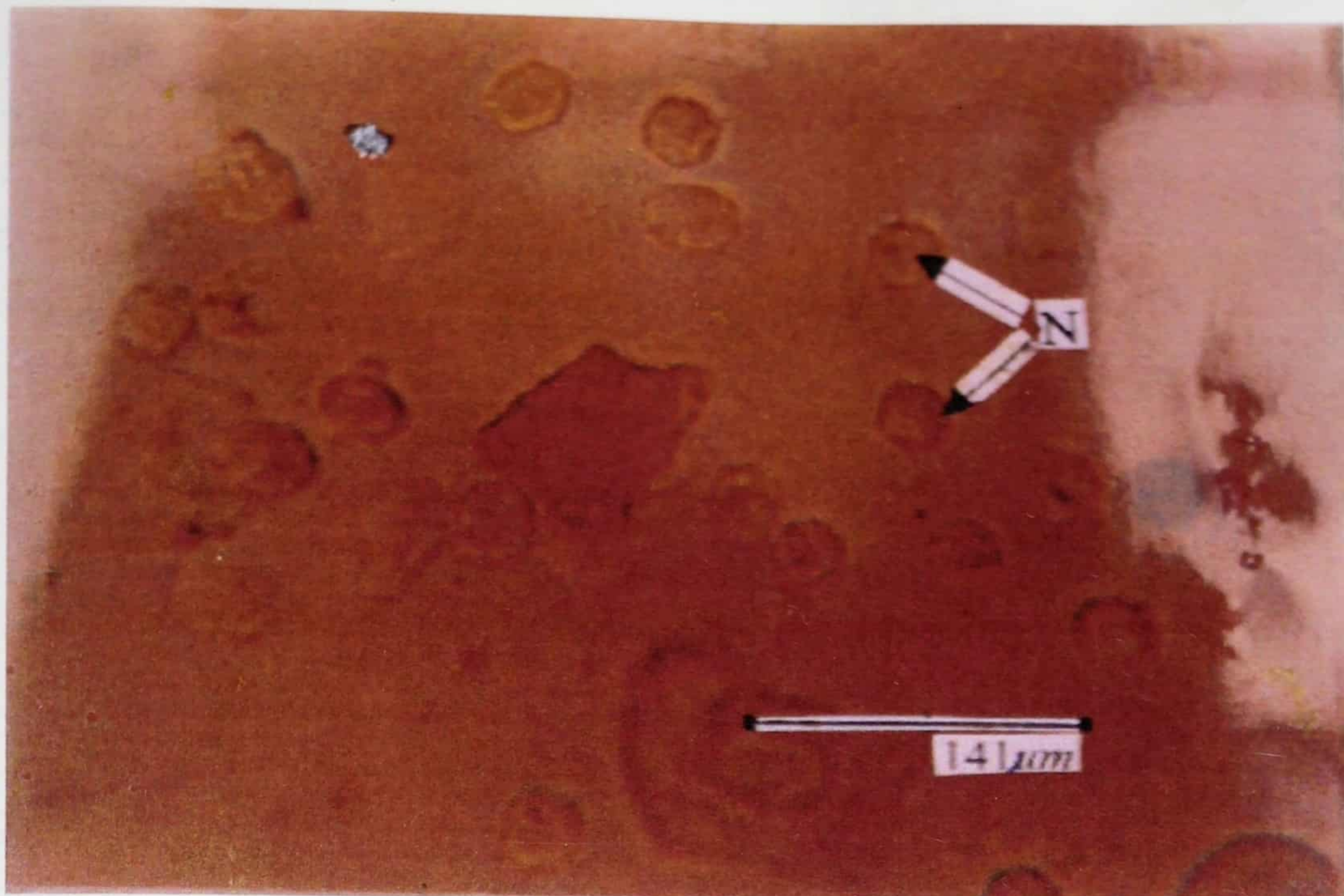


Plate 5

Photomicrograph of the vaginal smear of a **control animal (x128)**, at the late **Proestrus stage**. Note the presence of abundant nucleated cells (N).

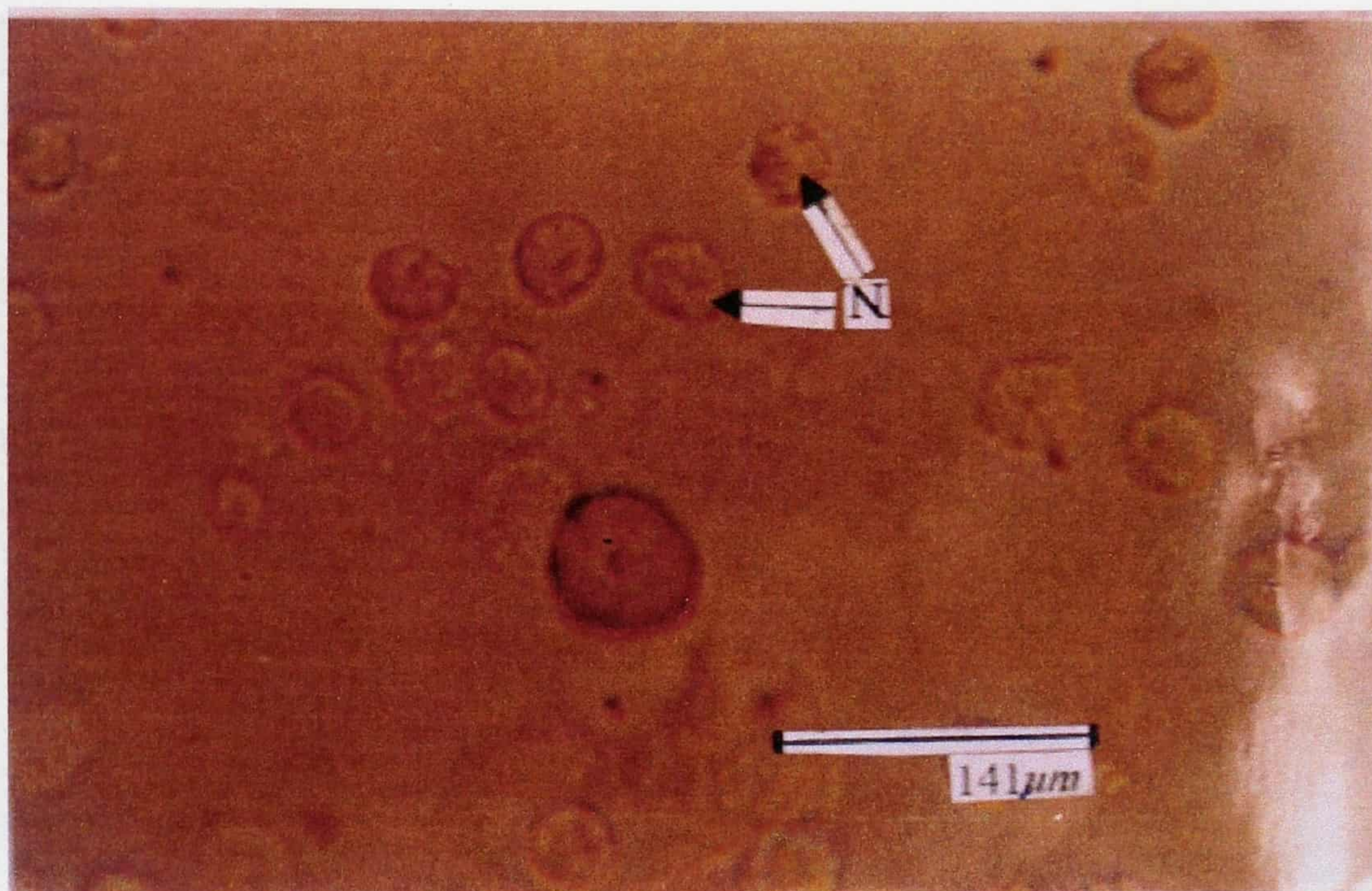


Plate 6

Photomicrograph of the vaginal smear of a **high dosed animal (x128)**, at the late **Proestrus stage**. Note the presence of abundant nucleated cells (N).

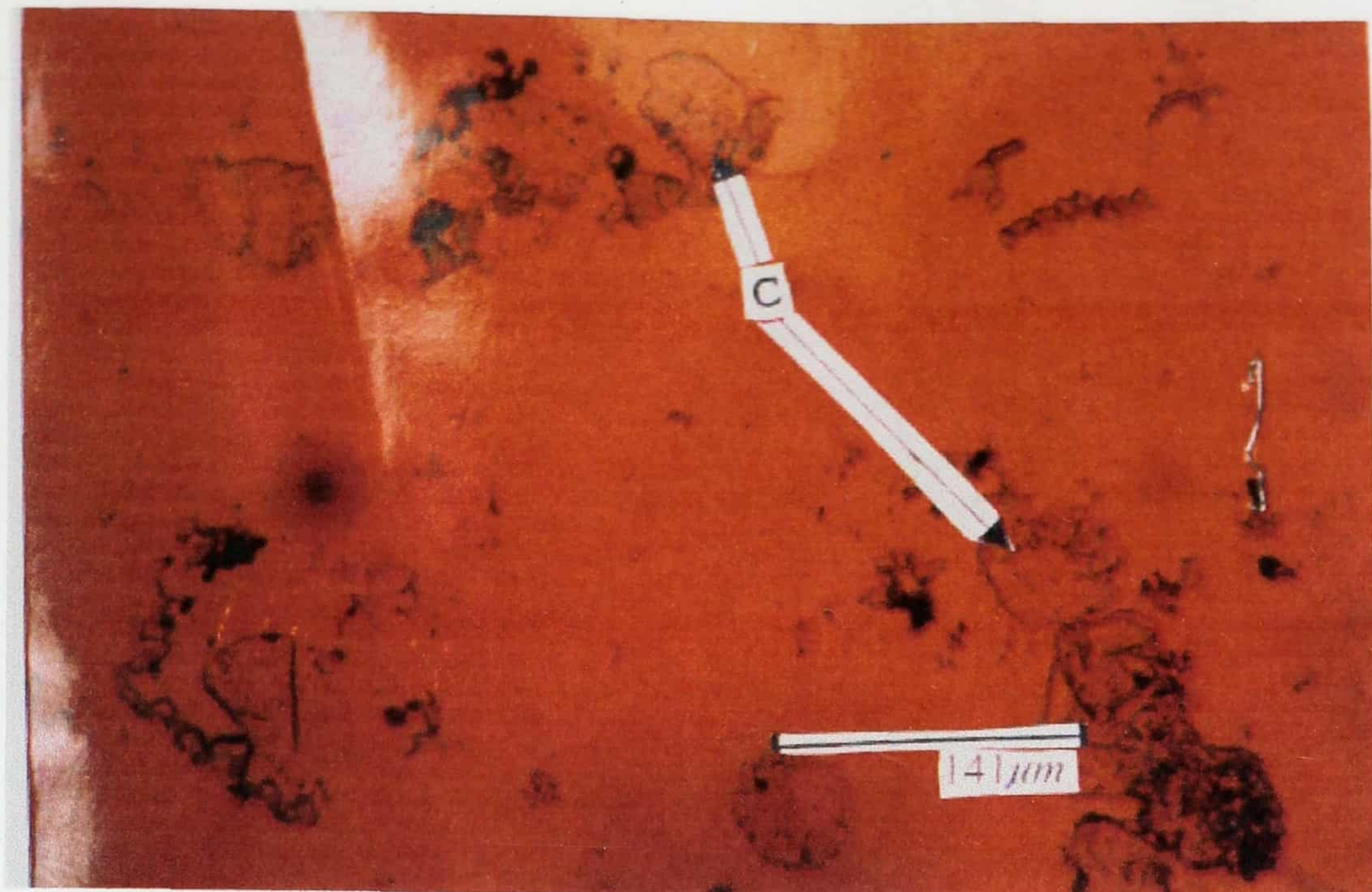


Plate 7

Photomicrograph of the vaginal smear of a **control animal (x128)**, at the **Oestrus stage**. Note the presence of abundant cornified cells (C).



Plate 8

Photomicrograph of the vaginal smear of a **high dosed animal (x128)**, at the **Oestrus stage**. Note the presence of abundant cornified cell (C).

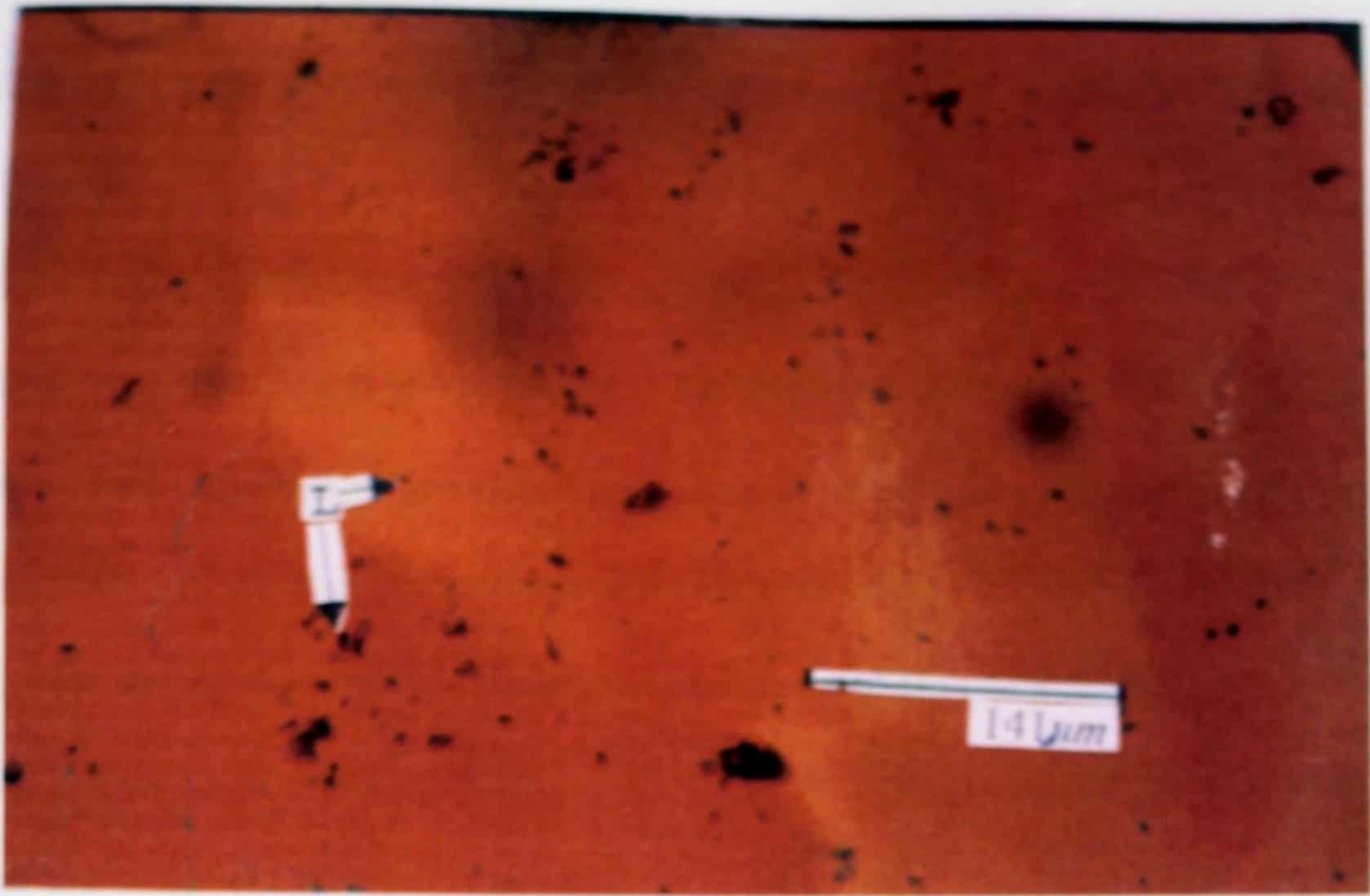


Plate 9

Photomicrograph of the vaginal smear of a **control** animal (x128), at the **Dioestrus** stage. Note the presence of abundant leucocytes. (L)

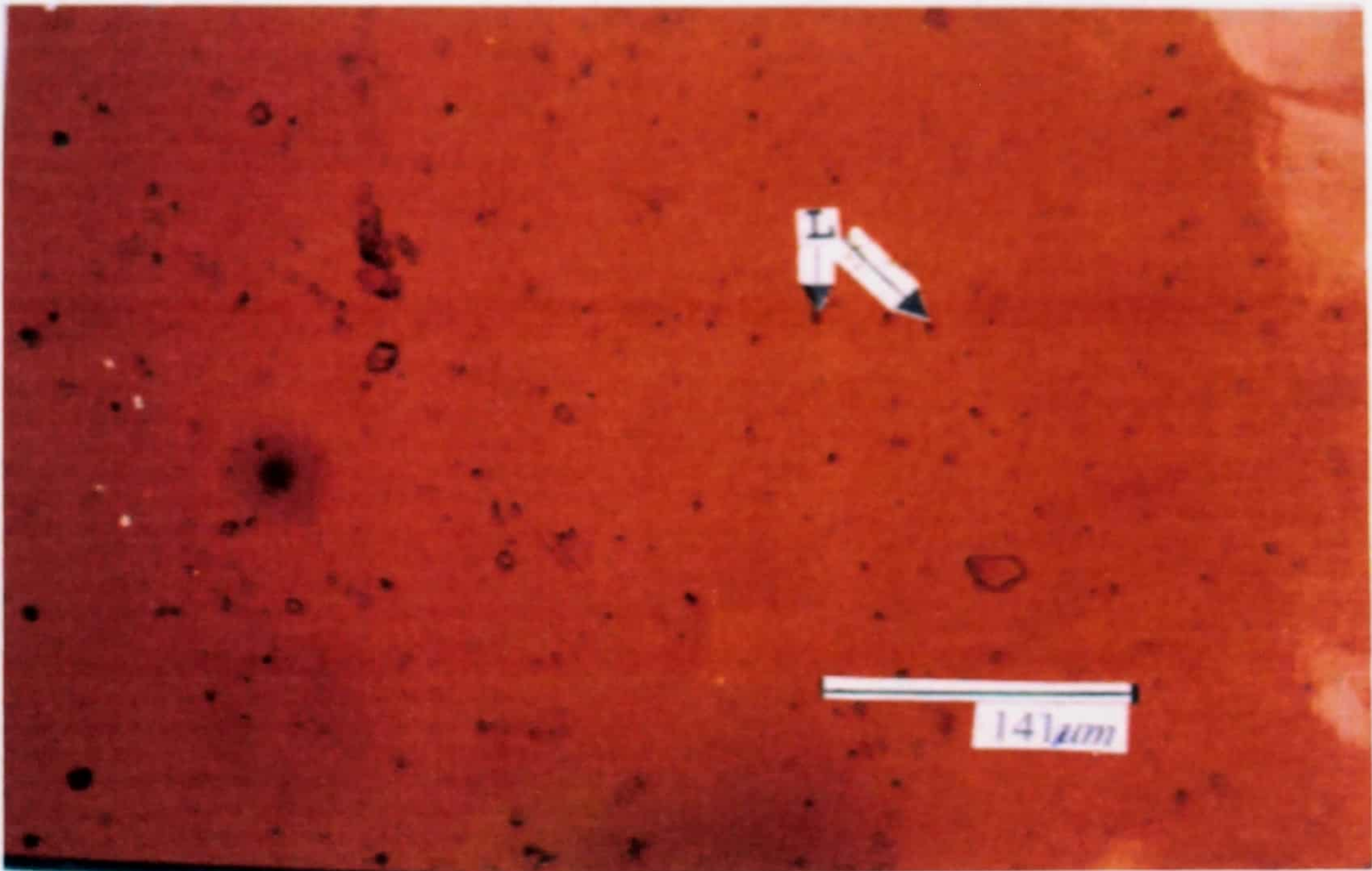


Plate 10

Photomicrograph of the vaginal smear of a **high dosed animal** (x128), at the **Dioestrus** stage. Note the presence of leucocytes (L)

4.2.2 Histological Studies of the Ovaries of the Animal of the Different Treatment Groups.

Microscopic examination of sections of the ovaries of the animals on the different treatments showed that almost all the stages of development of the ova were observed for animals in each of the different groups. There were no observable significant differences between the stages of development of ova in the ovaries of the different treatment groups, including those on the high dose of the extract and those of the control animals.

The number and nature of the lutein cells of the corpus luteum were almost the same in all the treatment groups. The Graafian follicles were also almost the same in all treatment groups including the controls.

The comparison of the histological sections of the ovaries of the animals on the different treatments showed no characteristic differences.

Plates 11 to 16 show the photomicrographs of ovaries treated with a high dose level of the extract of fruit of *Jatropha curcas* for 16 days and those of the control animals.

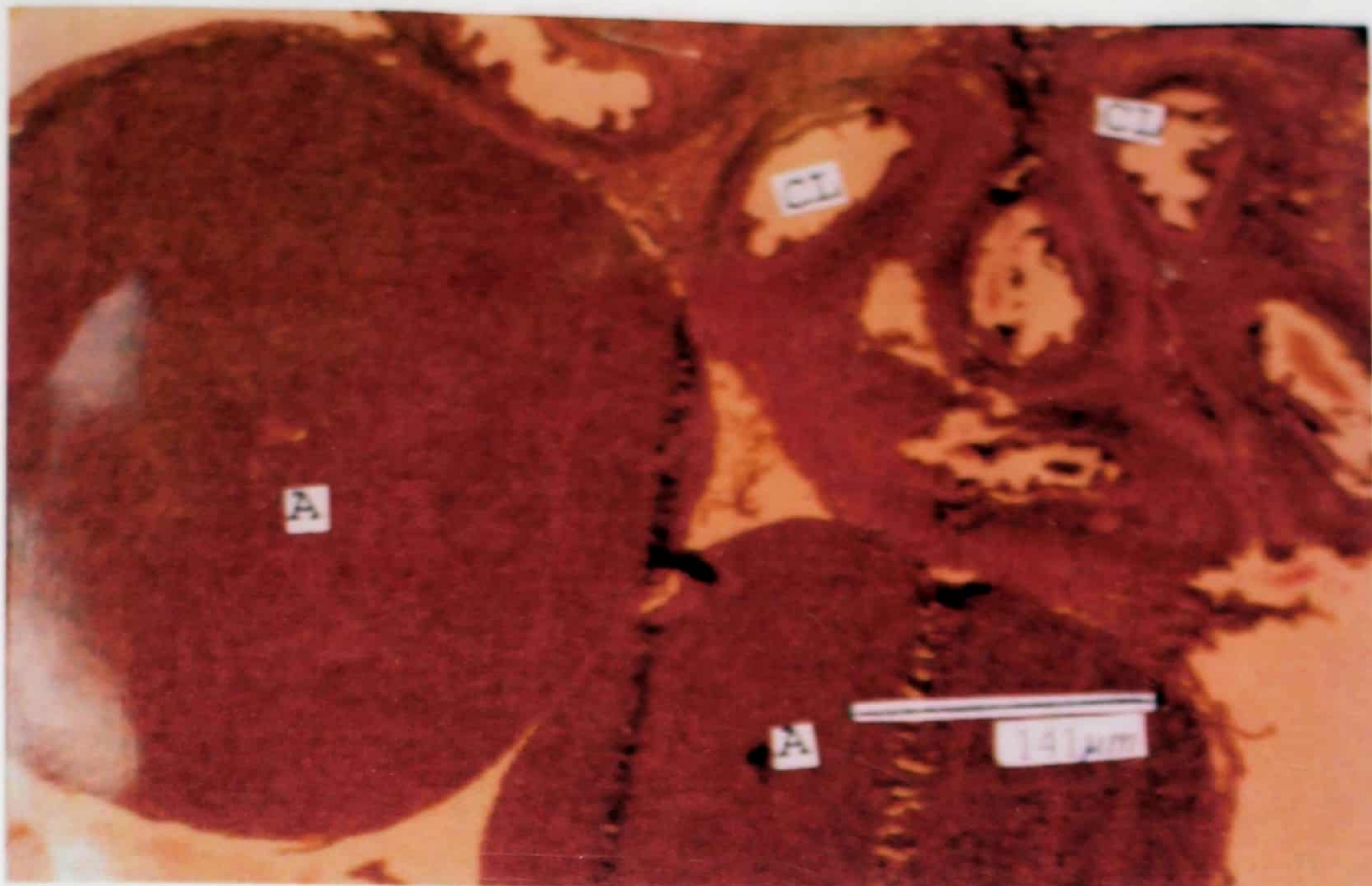


Plate 11

Photomicrograph of a section through the ovary of a **control** animal (x128), showing **corpora lutea (CL)** and **corpora albicans (A)**.

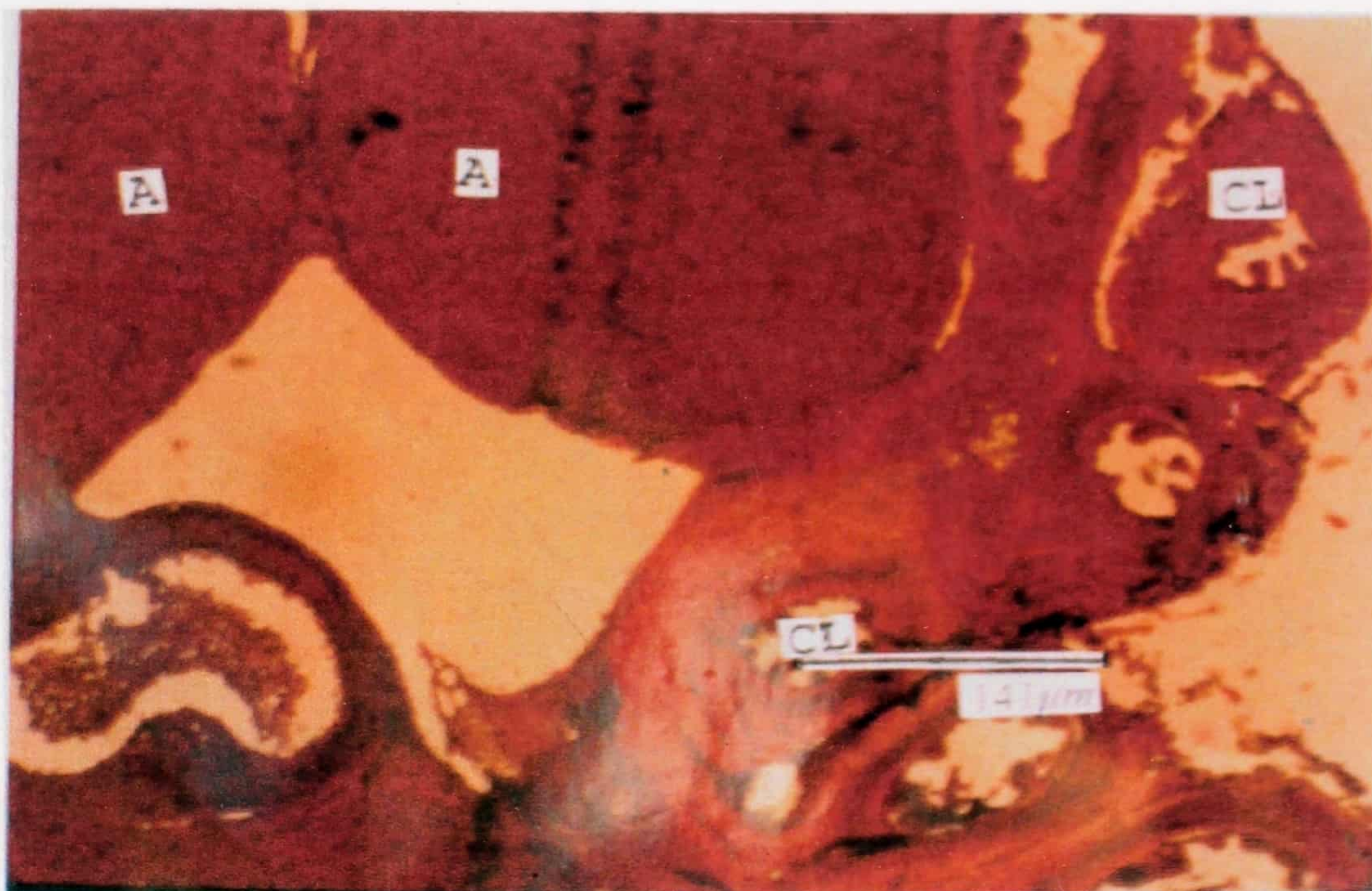


Plate 12

Photomicrograph of a section through the ovary of a **high dosed** animal (x128),

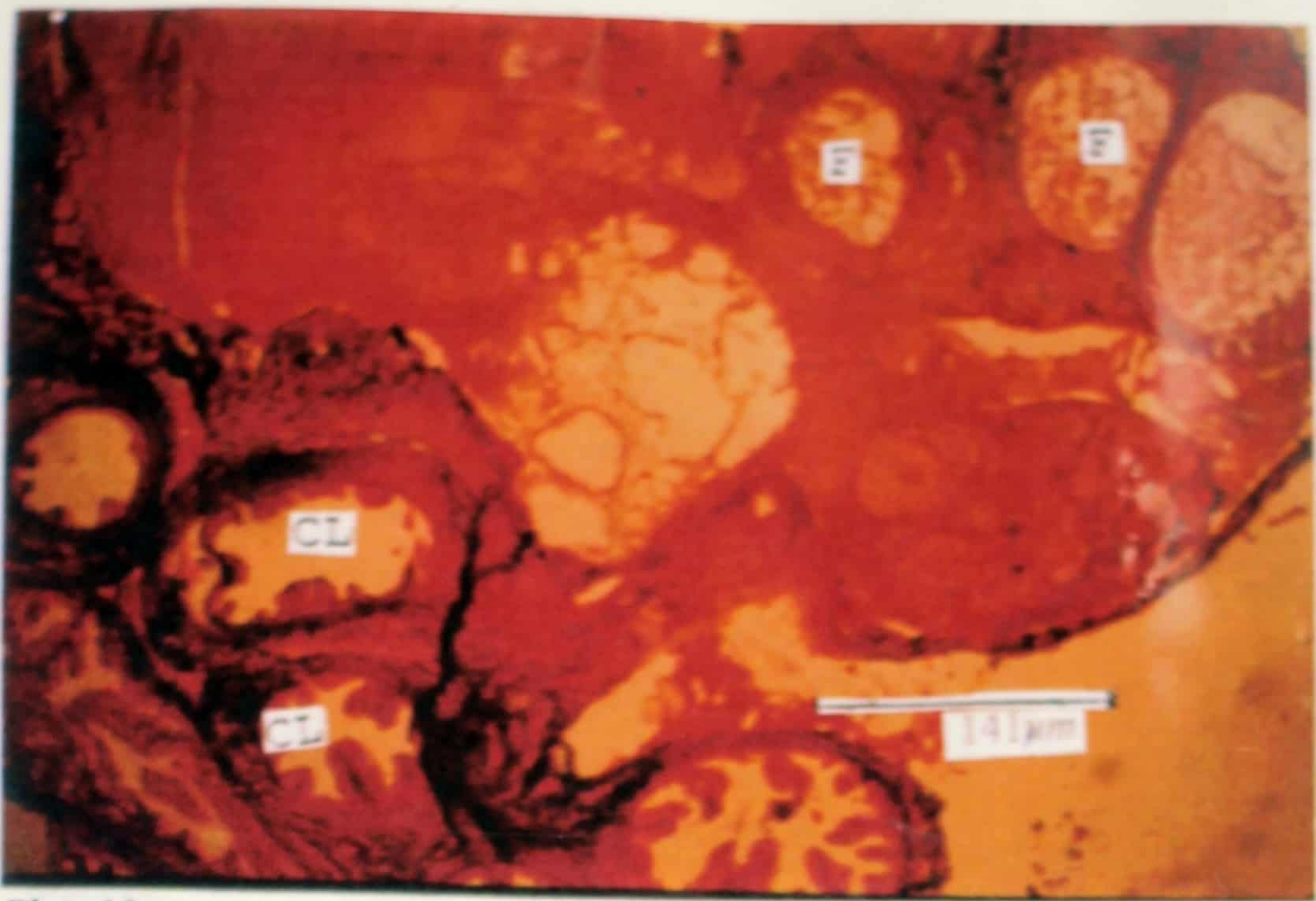


Plate 13

Photomicrograph of a section through the **Ovary** of a **control** animal (x128) showing **follicles (F)** and **corpora lutea (CL)**.

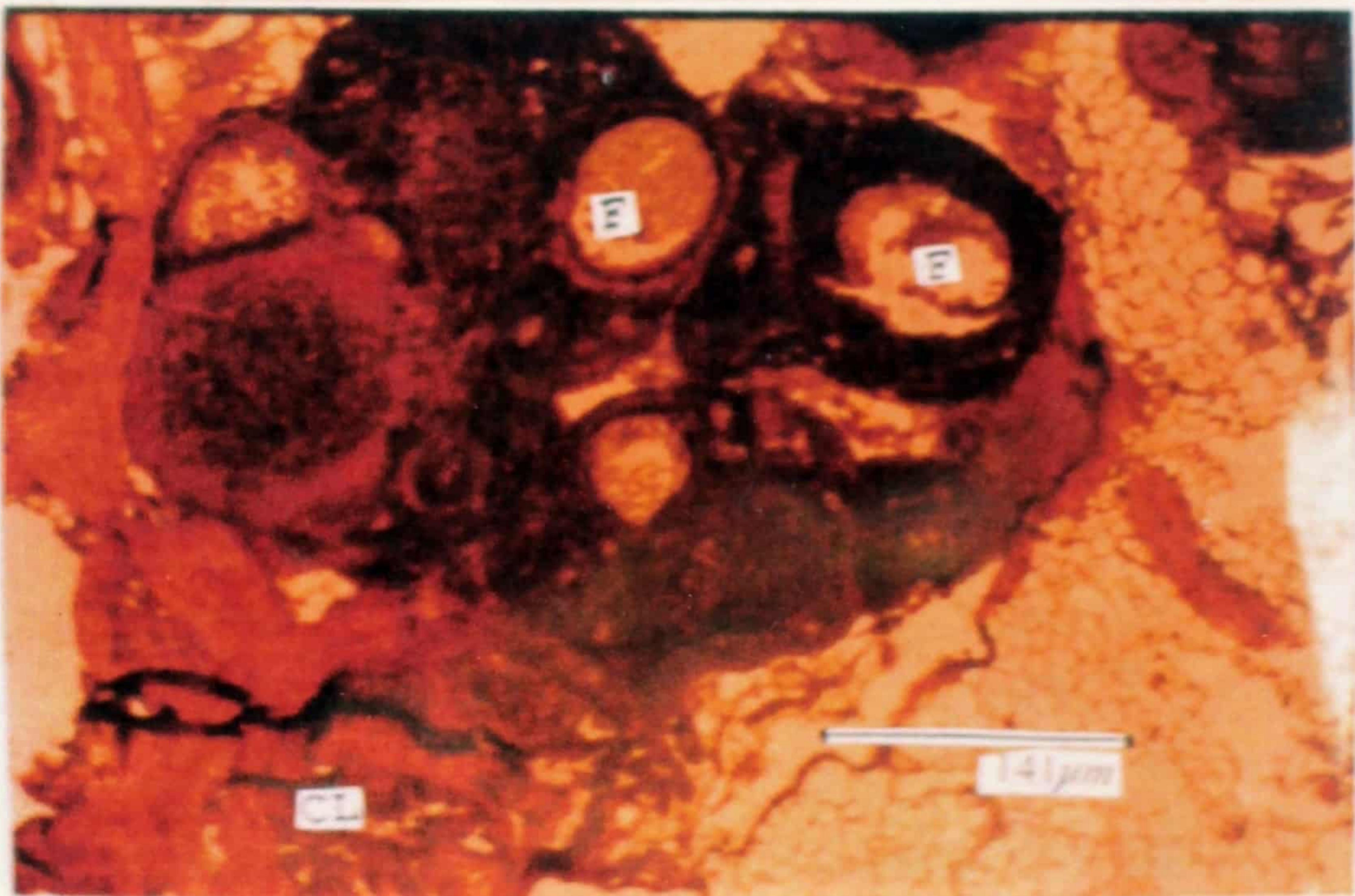


Plate 14

Photomicrograph of a section through the **Ovary** of a **high dose** animal (x128), showing **follicles (F)** and **corpora lutea (CL)**

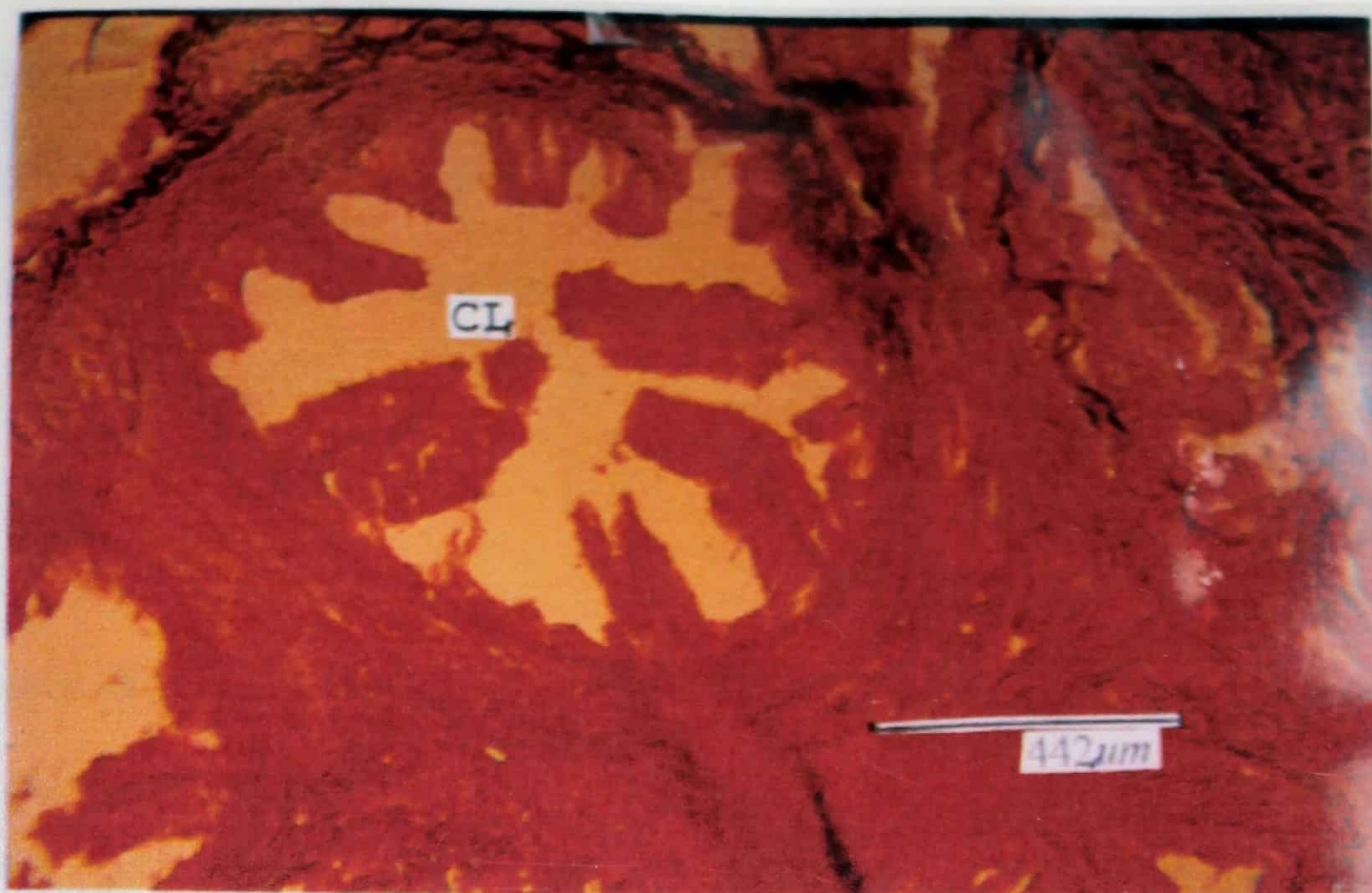


Plate 15

Photomicrograph of a section through the **Ovary** of a **control** animal (x400), showing **corpora luteum (CL)**

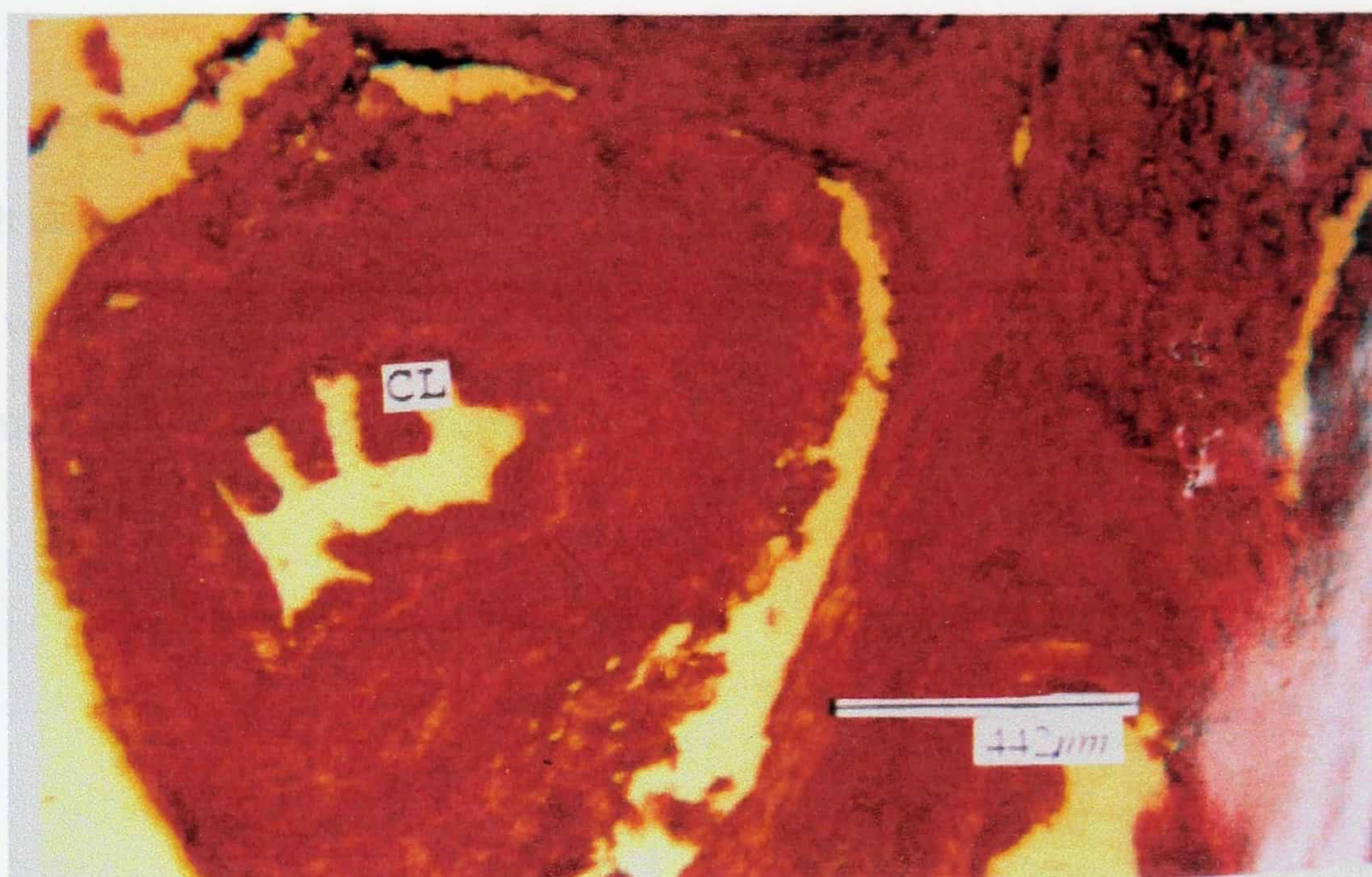


Plate 16

Photomicrograph of a section through the **Ovary** of a **high dose** animal (x400), showing a **corpora luteum (CL)**.

4.3 EFFECT OF THE EXTRACT OF *Jatropha curcas* ON THE OVULATION OF IN THE FEMALE ALBINO RATS

The number of corpora lutea represents the number of eggs ovulated and for that matter ovulation rate in the rats

Table 4.3

Mean number of corpora lutea in the ovaries of animals in the different treatment groups dosed for 16 days.

Treatment group	Dose level (g/kg)	Mean number of corpora lutea.
T ₀	0	10.25 ^a
T ₁	1.5	10.57 ^a
T ₂	2.0	10.83 ^a
T ₃	2.5	11.29 ^a

Differences in mean not significant when superscripts are the same.

The result showed that the extract had no effect on ovulation rate in the female albino rat as there were no significant differences in the mean number of corpora lutea in the different treatment groups and the control.

4.4 EFFECT OF THE EXTRACT OF *Jatropha curcas* ON POSITIVE MATING (PREGNANCY) IN THE FEMALE ALBINO RATS.

The Plate below (plate 17) shows positive mating seen in one of high dose animals.

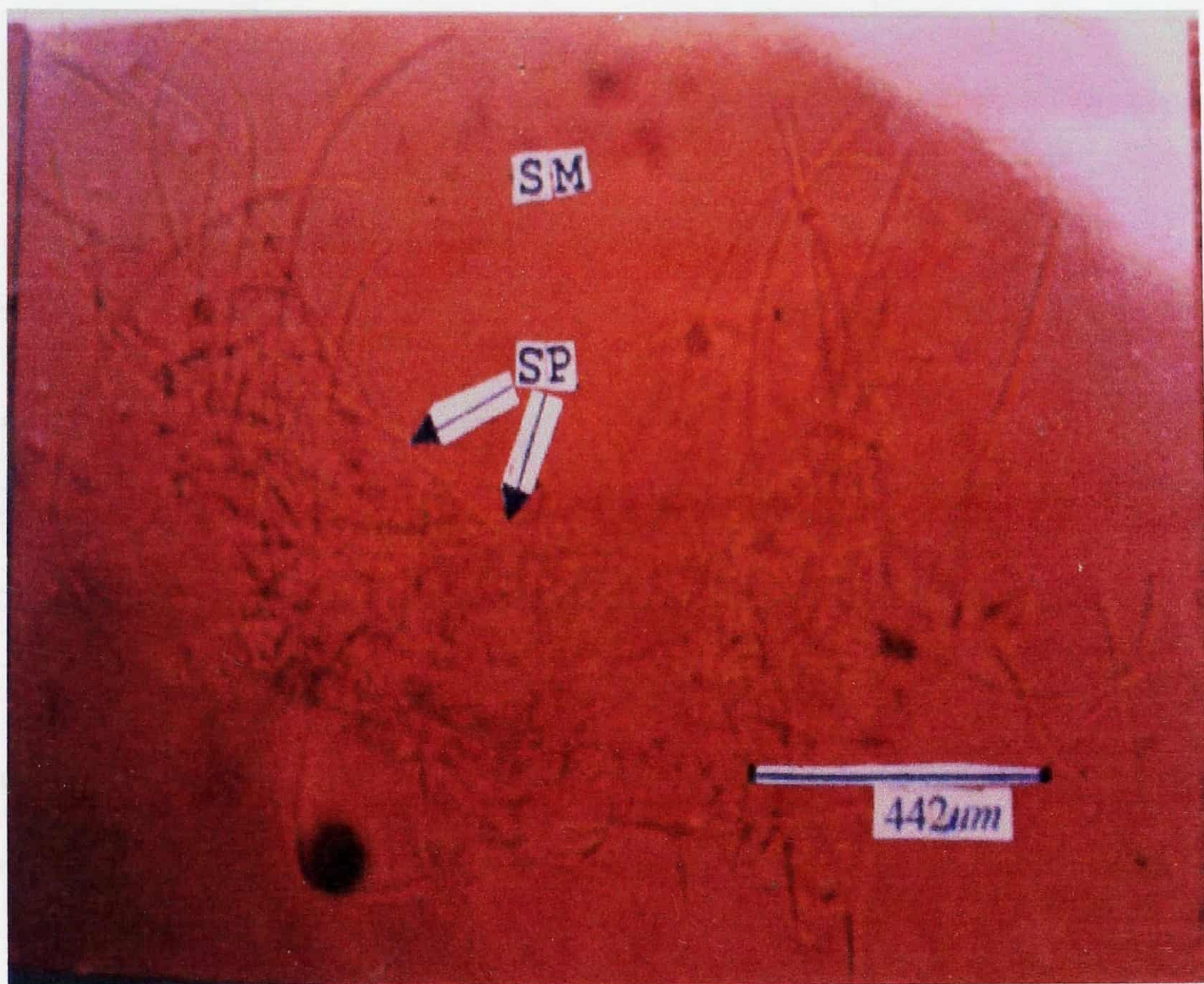


Plate 17

Photomicrograph of a vaginal smear (x400), showing **positive mating** or pregnancy, with **sperms (SP)** in the smear (**SM**).

Table 4.4

Number of rat pregnant per number of rats dosed with different dose levels of the extract.

Treatment group [Dose level (g/kg)]	Number pregnant per number dosed.	Mean fraction of pregnancy
T ₀ (0)	7/8	0.88 ^a
T ₁ (1.5)	6/8	0.75 ^a
T ₂ (2.0)	8/8	1.00 ^a
T ₃ (2.5)	7/8	0.88 ^a

Differences in means not significant when superscripts are the same

The comparison of the number pregnant per number dosed for the different treatment groups and the control shows no significant differences. The result indicates that the alcoholic extract of *Jatropha curcas* did not affect pregnancy or conception in the female albino rats.

4.5 EFFECT OF THE ALCOHOLIC EXTRACT OF FRUITS OF *Jatropha curcas* ON IMPLANTATION

The table below summarises the results of the effect of the extract on implantation.

Table 4.5

Mean number of implantation sites of in the animals the different treatment groups of the extract.

Treatment group	Dosage Level (g/kg)	Mean number of implantation sites.
T ₀	0	9.2857 ^b
T ₁	1.5	5.1667 ^a
T ₂	2.0	4.8750 ^a
T ₃	2.5	2.5714 ^a

Differences in means are significant when superscripts are different.

The results showed that the extract has a significant effect on implantation in the female albino rats. This is because the number of implantation sites recorded in the control animals (given distilled water only) was significantly higher than those of the animals dosed with the extract. The lowest number of implantation sites was recorded in the highest dosed animals. The second lowest number of implantation sites was recorded in the medium dosed animals and third the highest number of implantation sites was recorded in the low dosed animals.

The pictures below show implantation sites in dissected animals.

The picture below Plate (18) shows the uterine horns of an albino rat. Notice the sites of implantation. The animal was given distilled water for sixteen consecutive days before they were dissected.

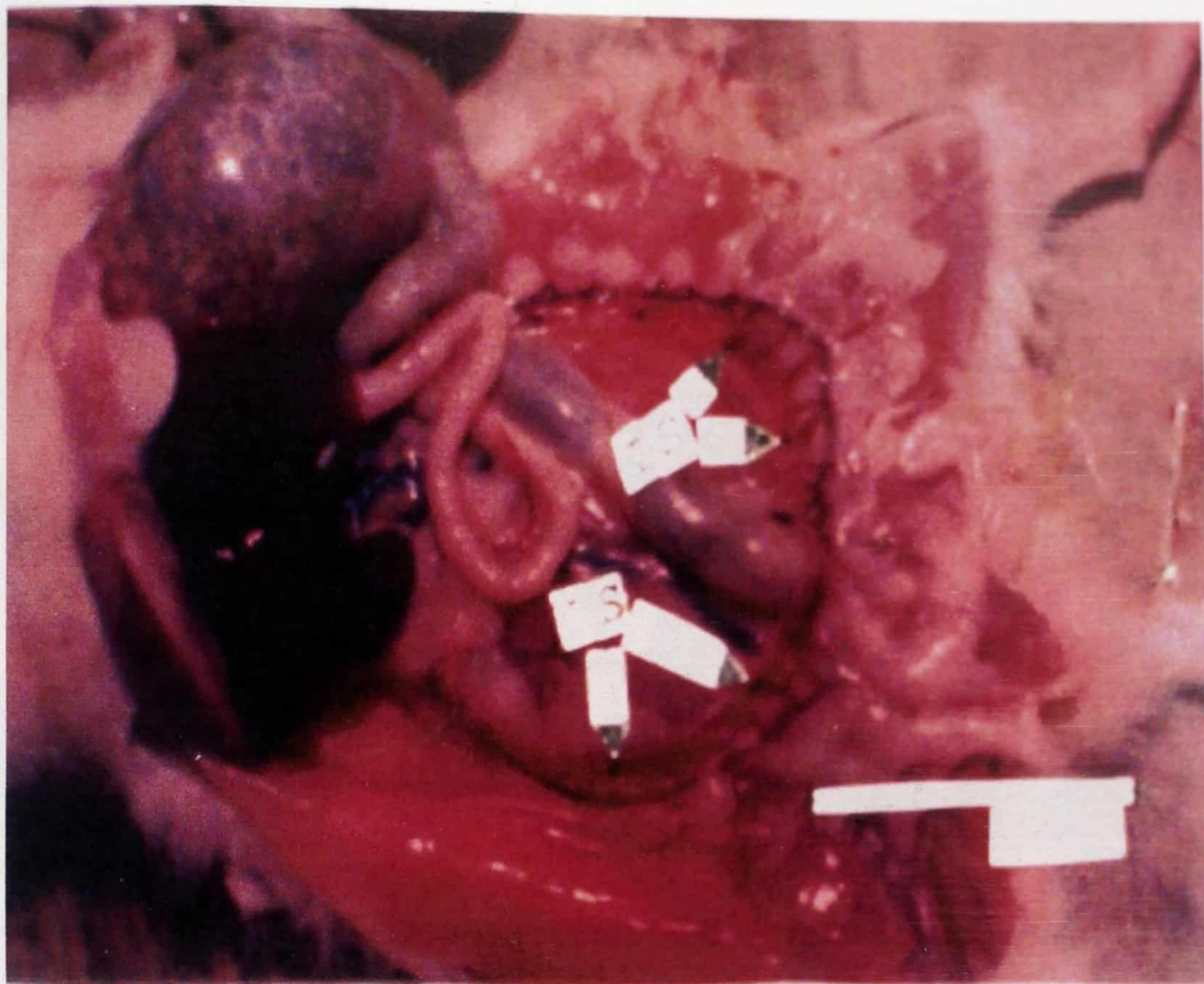


Plate 18 Photograph of a control animal (x0.9), showing implantation sites (IS)

The picture below Plate (19) shows the uterine horns of an albino rat. Notice the sites of implantation. The animal was given 2.5 g of the alcoholic extract of fruits of *Jatropha curcas* for sixteen consecutive days before they were dissected.

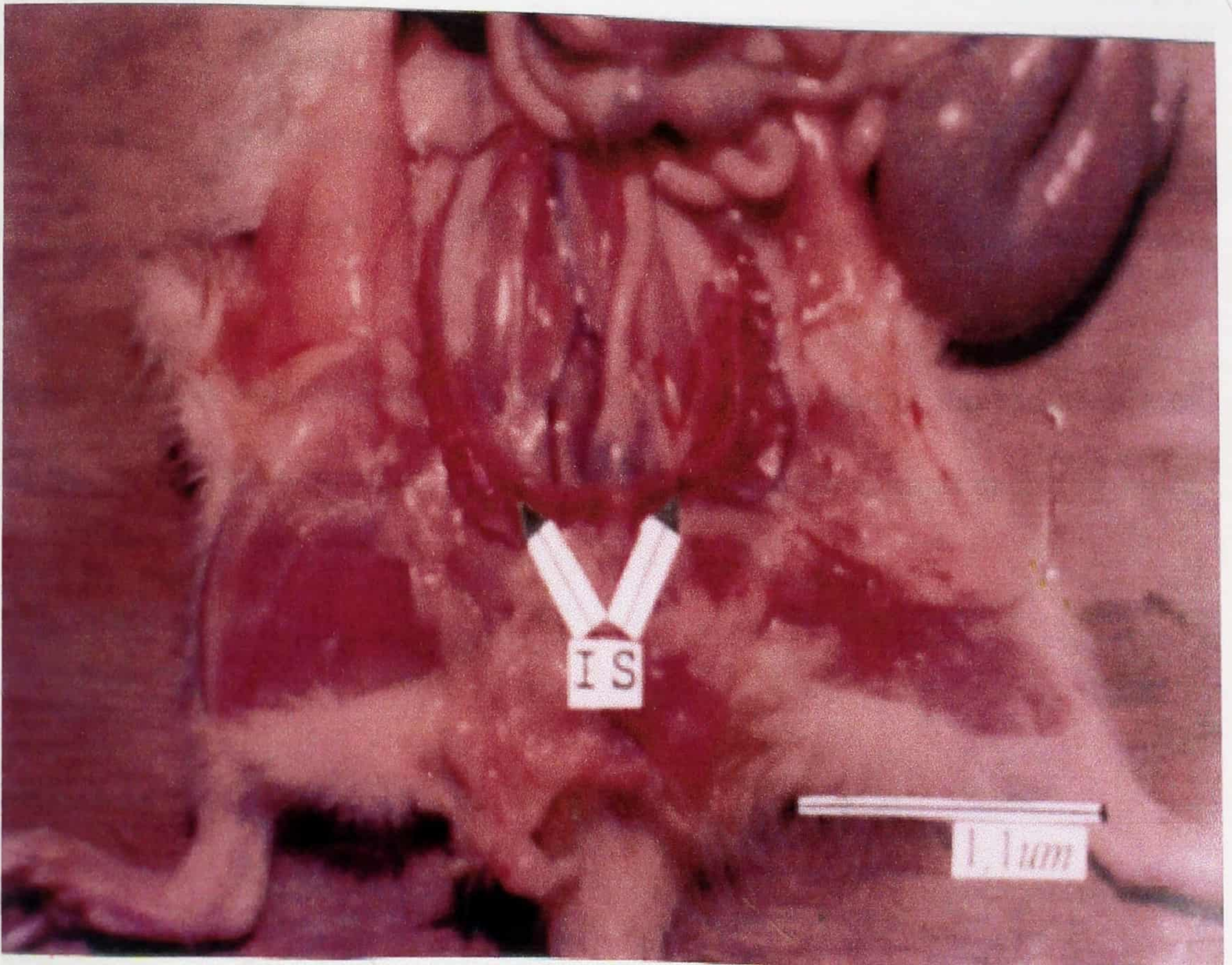


Plate 19

Photomicrograph of uterus of a **high dose** animals (x0.9), showing **implantation sites (IS)**

4.5.2 Histological examination of the uteri of the rats.

Microscopic examination of section of the uteri of the animals showed that there were no significant characteristic differences among the sections of the uterus of the animals on the different treatments and that of the control. The normal layers of the endometrium were observed for the animals on the treatment and the control.

Plate 20 and 21 illustrate the layers of endometrium of the uterus at low magnification. It shows the muscular wall, the myometrium (**M**) and the relatively thin endometrium consisting of the stratum basalis (**B**) and the stratum functionalis (**F**) consisting of the stratum spongiosum (**S**) and stratum compactum (**C**)

Plate 22 and 23 illustrate the endometrium at a higher magnification. It clearly shows the stratum spongiosum (**S**) and the stratum compactum (**C**) of the stratum functionalis.

There were no characteristic differences between the layers of the endometria of the high dosed animals the medium dose animals, the low dose animals and the control animals.

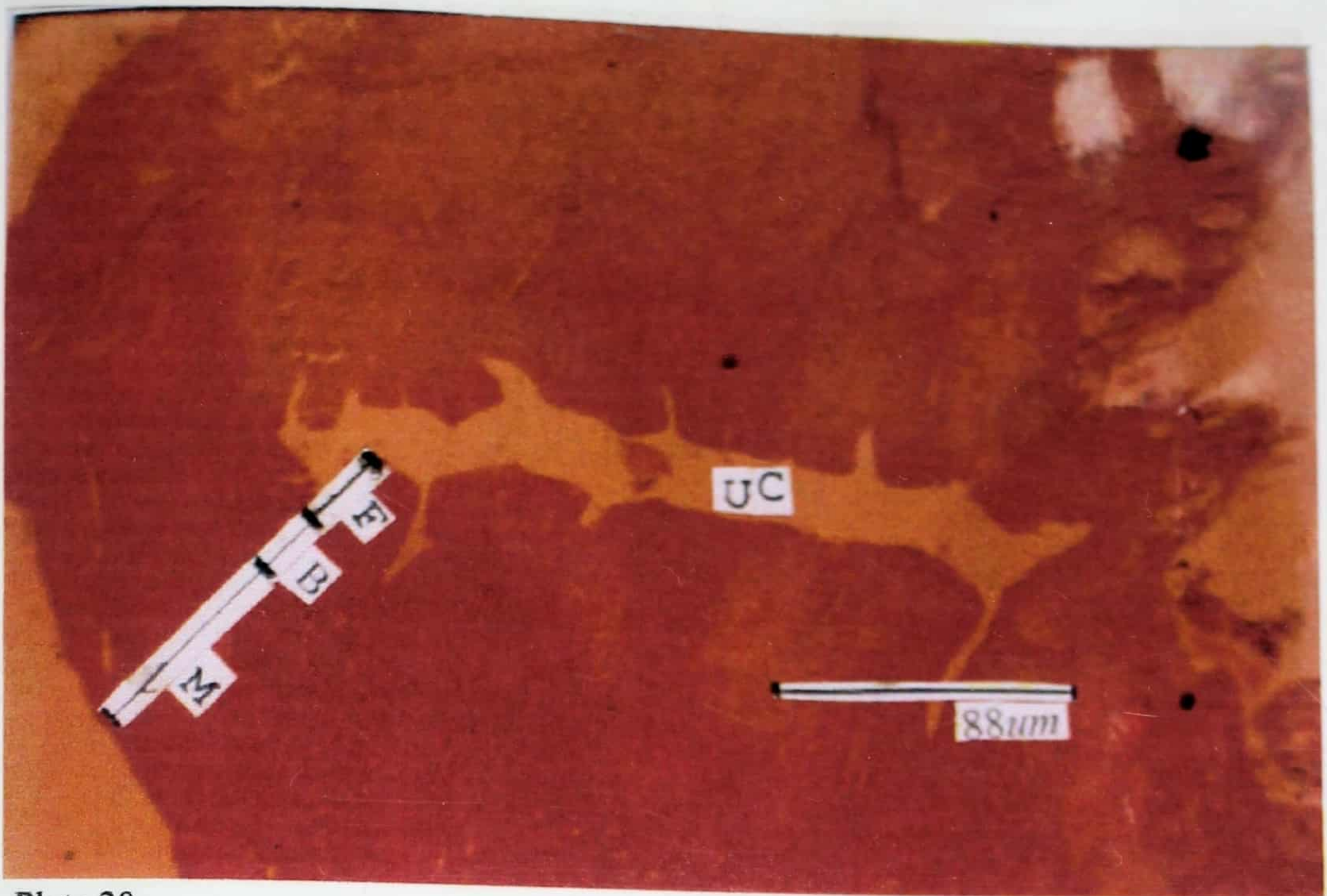


Plate 20

Photomicrograph of section through the uterus of a **control** animal (x80), showing the uterine cavity (UC) stratum functionalis (F) stratum Basalis (B) and myometrium (M)

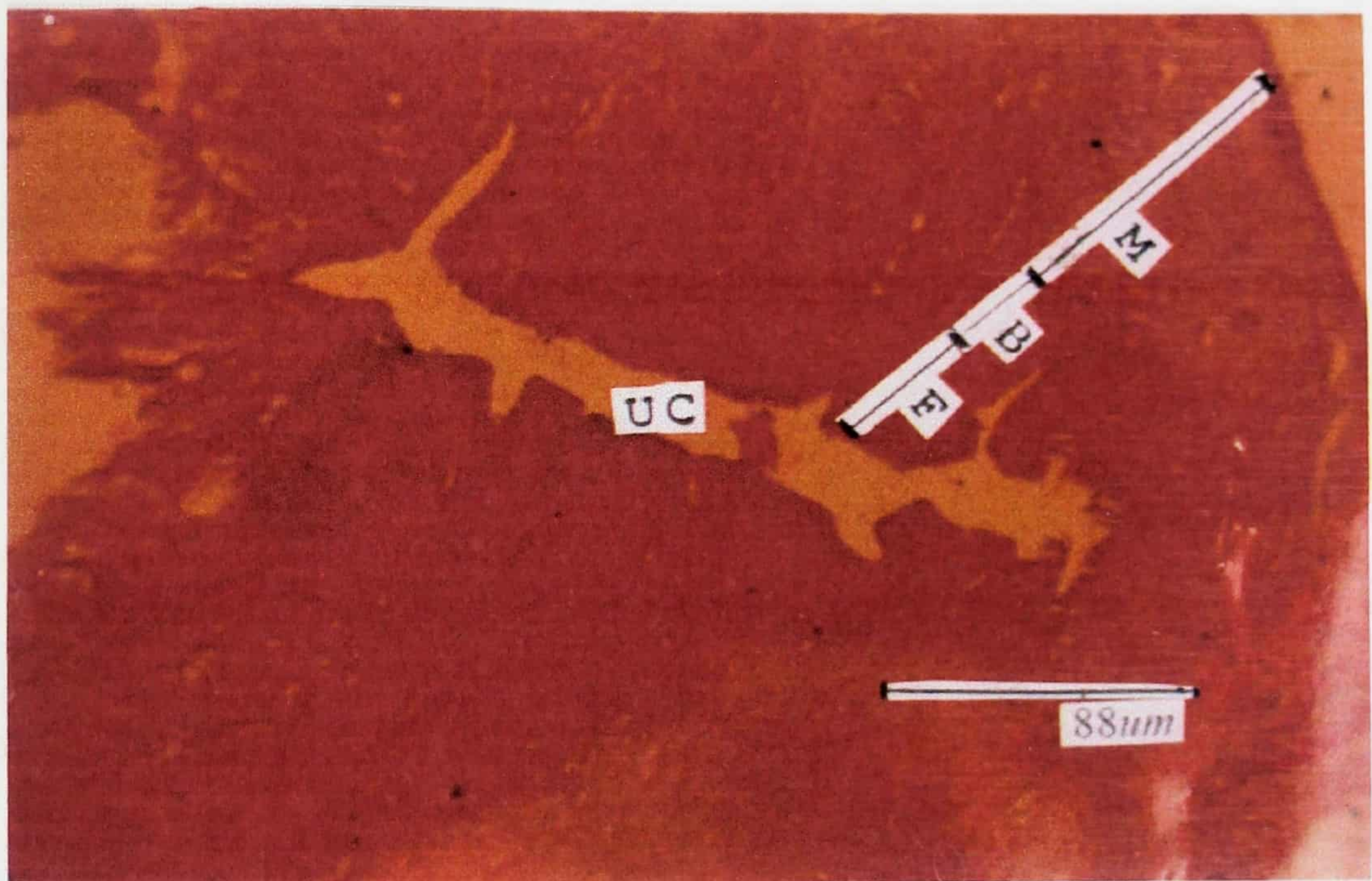


Plate 21

Photomicrograph of section through the uterus of a **high dose** animal (x 80), showing the uterine cavity (UC) stratum functionalis (F) stratum Basalis (B) and myometrium

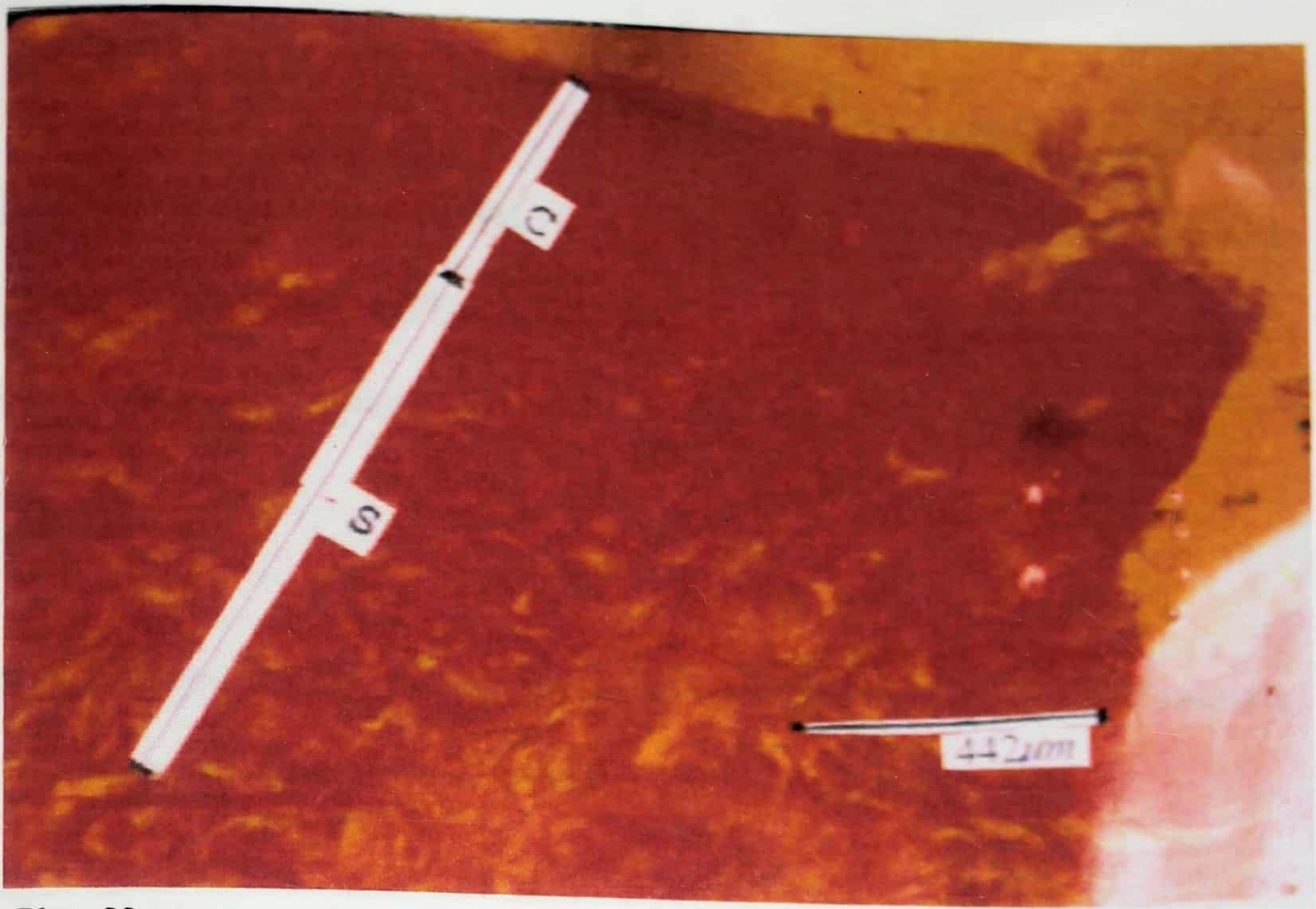


Plate 22

Photomicrograph of section through the endometrium of a **control** animal (x 400), showing stratum spongiosum (S) and stratum compactum (C) of the stratum

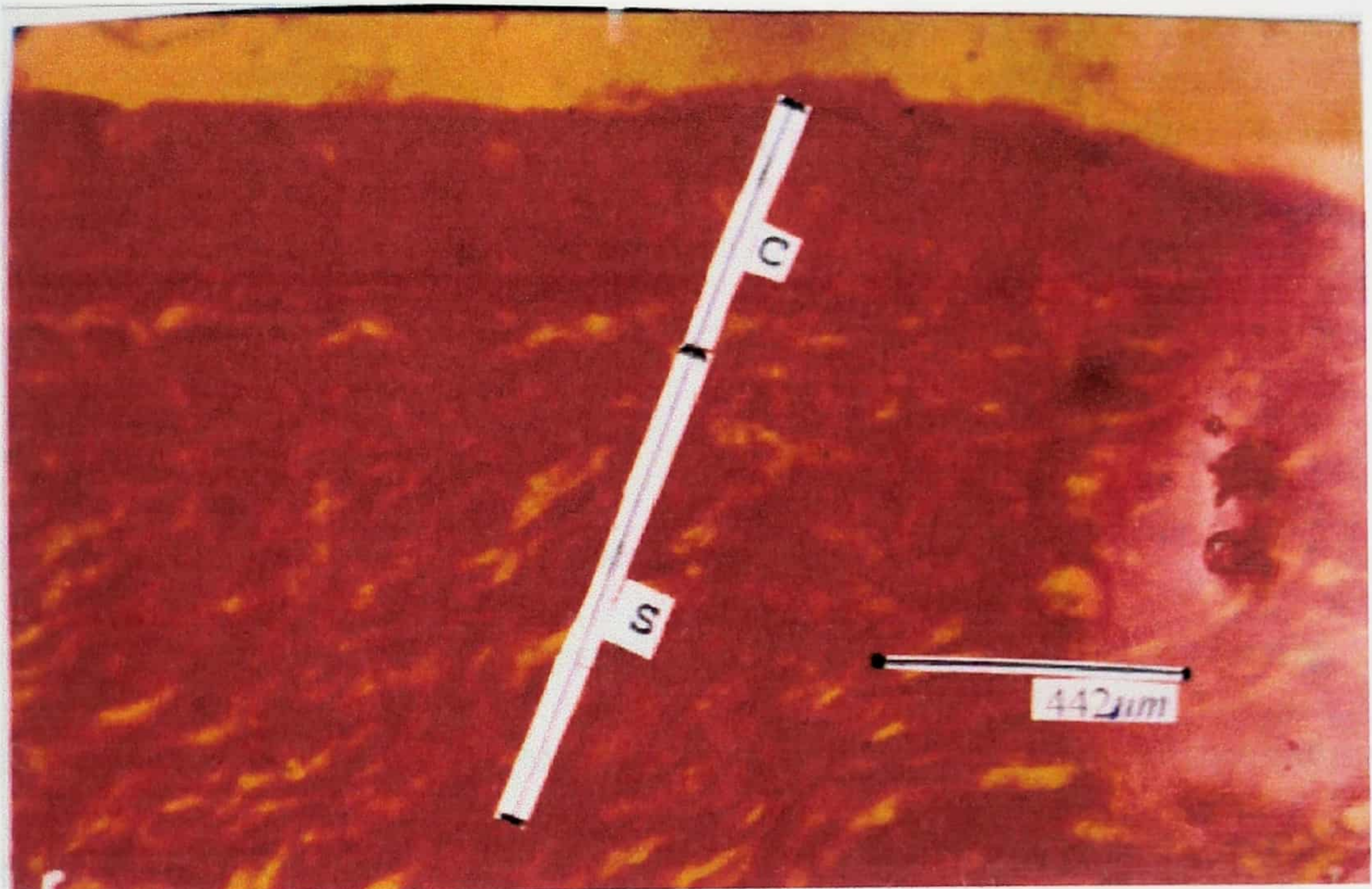


Plate 23

Photomicrograph of section through the endometrium of a **control** animal (x400)

Showing stratum spongiosum (S) and stratum compactum (C) of the stratum

4.6 EFFECTS OF THE ALCOHOLIC EXTRACTS OF FRUITS OF *Jatropha curcas* ON OVA WASTAGE OR FOETAL LOSS (ABORTION)

The effect of *Jatropha curcas* on ova wastage is shown in Table 4.6 below.

Table 4.6
Mean number of corpora lutea, normal foetuses removed and ova wasted in animals dosed with the different concentration of the extract of *Jatropha curcas*

Treatment group (Dose level)	Mean numbers of Corpora lutea	Mean number of foetuses removed	Mean number of ova wasted
T ₀ Distil water	9.50	9.00 ^b	0.50 ^d
T ₁ 1.5 g/kg	9.75	7.00 ^b	2.75 ^d
T ₂ 2.0 g /kg	9.35	3.50 ^a	5.86 ^e
T ₃ 2.5 g/kg	9.14	2.8 ^a	6.29 ^e

Differences in means are significant when superscripts are different.

The results showed that the extract could cause the loss of the implanted foetuses. The highest foetal loss was seen in the highest-dose animals, followed by the medium, then the low-dosed animals. Statistically, the mean number of ova wasted in the control was significantly less ($P < 0.05$) from that of the highest dosed animals.

4.7. EFFECTS OF THE ALCOHOLIC EXTRACTS OF FRUITS OF *Jatropha curcas* ON WEIGHT OF THE FEMALE ALBINO RATS

The results shown in table 4.7 below indicated that the animals did experience significant changes in weight when the extract was administered to them.

Table 4.7

Mean Total changes in weight (g) of female rats given different dosages of extract.

Treatment group (Dose)	Weight (g)			
	Day 4	Day 8	Day 12	Day 16
T ₀ (0 g /kg)	1.88 ^a	4.38 ^c	5.63 ^c	6.25 ⁱ
T ₁ (1.5 g /kg)	-1.88 ^a	0.03 ^{b c}	1.40 ^{e f}	-3.60 ^{h i}
T ₂ (2.0 g /kg)	-1.88 ^a	-1.94 ^{b c}	-7.74 ^{b c}	-9.64 ^h
T ₃ (2.5 g /kg)	- 2.53 ^a	-8.47 ^b	-17.34 ^d	-25.87 ^g

Differences in means are significant when superscripts are different.

The effect of *Jatropha carcus* administration on body weight changes in the albino rats on the different treatments is shown in Table 4.7. The results show clearly the following:

1. Animals given the extract lost weight during the period of treatment. In contrast, controls gained weight.

2. The loss of weight was highest in the animals given the highest dose of the extract of *Jatropha curcas*.

4.8 Toxicological effect of the alcoholic extract of fruits of *Jatropha curcas* on female of albino rats.

More deaths were recorded in the animals given the high dose level of extract than in the other dose levels. No death was recorded in the control animals.

In the experiment to verify the effect of the extract on the length of oestrous cycle, 2 deaths were recorded in the high – dosed (2.5 g/kg) animals whereas 1 animal each died from the medium dosed group (2.0 g/kg) and the low dose group (1.5 g/kg). See Appendix 2, Table Ap.2) Also, in the experiment to determine the effect of the extract on implantation, 1 animal died in the high dose group, but none died in the lower doses and the control groups. (See Appendix 4, Table Ap. 4). Statistically, these values were not significantly different from one another.

CHAPTER FIVE

DISCUSSION

5.0 DISCUSSION

The indications from the results of the work show *Jatropha curcas* as a fertility control agent.

There were no significant differences between the mean length of oestrous cycle of the rats given the extract (treated animals) and those who were not given any of the extract (control). The mean length of oestrous cycles of the treated animals (low, medium and high) did not significantly differ from one another (Table 4.1).

The mean oestrous cycle length of 4.40 days obtained for the control animals falls in between the one recorded by Bedu-Addo (1993) and that of Asdell (1946). Bedu-Addo (1993) recorded 4.26 days while Asdell (1946) recorded 4.80 days as the mean oestrous cycle lengths for the untreated rats.

The fact that the length of oestrous cycle recorded for the animals dosed were neither different from those of the control nor different from each other indicates that the alcoholic extract of fruits of *Jatropha curcas* did not affect the length of the oestrous cycle of the female albino rat.

Hormones, secreted in a complex process-involving interaction between the hypothalamus, anterior pituitary and ovaries control the activities of the oestrous cycle (recruitment, growth and maturation of follicles). The major hormones involved are the gonadotrophin releasing hormone (GnRH) produced by the hypothalamus, follicle stimulating hormone (FSH) and Luteinizing hormone (LH) produced by the anterior pituitary and the ovarian hormones:- oestrogen and progesterone. Also involved at

various levels of the oestrous cycle are neurotransmitters, prostaglandin and ovarian peptides (Bedu-Addo, 1993). The alcoholic extract of *Jatropha curcas* did not interfere with the hormonal control of the ovarian activities. This explains why the ovarian length, (the time taken for the cycle of activities of the oestrous cycle to occur) was not affected.

The oestrous cycle length for almost all the animals was either 4 days or 5 days. During the experiment however, quite a long oestrous cycle length was recorded for the animal with identification number T1R4; (Appendix 2, Table Ap2). This happened once and the animal died. This rather unfortunate incidence could not be due to the effect of the extract of *Jatropha curcas*. Excessive stimulation of the vaginal smear during the taking of smear may upset the oestrous cycle and produce pseudopregnancy with a duration about 12 –16 days before the resumption of a normal oestrous cycle.

The results indicate that the alcoholic extract of *Jatropha curcas* used did not affect the development of ova in the albino rats. The recruitment, growth and maturation of follicles into ova are greatly controlled by hormones, such as FSH and LH. FSH is known to stimulate the production of oestrogen which brings about the rapid growth and cornification of the vaginal epithelium. The regular follicular cycles associated with spontaneous ovulation are reflected in the predictable changes in plasma oestrogen and progesterone and, consequently changes in the vaginal epithelium. Examination of a vaginal smear of a spontaneous ovulator therefore provides an indication of the normal state of the animals. The observation of normal pattern of vaginal epithelial cells in the smears was an indication of normal production of hormones (oestrogen and progesterone) and for the normal recruitment, growth and development of follicles into ova. Of all the

animals treated with the different dose levels of extracts, all the normal vaginal smear patterns were observed. (Plates 3 to 10)

The regular histological changes in the vaginal epithelium were the same for all the experimental animals (high, medium, low, control). Further more, microscopic examination of the sections of all the ovaries of the treated animals and the control, showed all the observable stages of development of ova in the ovaries. The above observation suggests that ova development was not affected, by the extract of *Jatropha curcas*.

Hormones control the development of ova. The release of these hormones can be inhibited by chemicals (Barracclough and Sawyer, 1955) and endogenous opiate peptides (Kinoshita *et al.*, 1980). However the presence of these chemicals in the fruits *Jatropha curcas* is not documented. Ewing in 1983 showed that a variety of endocrine tissues, including the pituitary gland respond to prostaglandin. This suggests that the release of growth hormone would be impaired if the prostaglandin fail to stimulate the anterior pituitary. The extract therefore did not affect the production of prostaglandin and that was why, the anterior pituitary gland produced the normal hormones for the normal recruitment, growth and maturation of follicles. *Jatropha curcas* does not contain gonadotrophin-inhibiting chemicals such as morphin and so the extract did not interfere with the normal function of gonadotrophin hormones. The hormones therefore promoted protein synthesis and affected the distribution of all categories of nutrients, resulting in the normal development of the ova. The absence of marked dissimilarities in the cells and tissues in the histological sections of the ovaries of the animals on the different

treatment gave an indication that the extract does not interfere with the development of the ova.

The present results indicate that the alcoholic extract of *Jatropha curcas* used did not affect the ovulation rate of the albino rat. The mean number of corpora lutea obtained in the four treatment groups showed no significant differences statistically. The presence of corpora lutea in the ovaries in all the dosed animals suggests that the extract did not stop the ovarian cycles and ovulation.

This result was similar to those of Goonasekera and others in 1995 who did not also observe any luteolytic effect of the extract *Jatropha curcas*.

The same explanation for no effect on oestrous cycle and ova development can also be given to why the extract had no effect on ovulation. This is because all these events are controlled by the same hypothalamus-pituitary-ovaries hormone relationship. The extract did not have the required concentrations of the chemicals that could interfere with the hormonal processes leading to ovulation. LH brings about ovulation. LH secretion is reported to be inhibited by some of the newly discovered endogenous opiate (Kinoshita *et al.*, 1980), however, the effect is not due to direct inhibition of LH release from the pituitary (Cicero, *et al.*, 1979) but to the prevention of GnRH secretion (Blank and Roberts, 1982). The uninhibited pituitary secretes FHS which causes the graafian follicles to develop and also causes the ovary to produce oestrogen. The oestrogen stimulates the pituitary to produce LH for ovulation to take place.

The results show that the different dose levels of the alcoholic extract of *Jatropha curcas* used did not interfere with successful mating. When the dosed rats and the control were paired (mated), the number of pregnancies recorded for the dosed

animals were not significantly different from that of the control. There were also no significant differences in the number of positive mating among the treated animals. This results agree with the results of Goonasekara *et al.*, (1995) who recorded no significant differences in the number of pregnancies occurring in dosed and control rats.

It is assumed that fertilization will take place if a sperm is found in the uterus of a rat, which is in its oestrous phase and for that matter ovulating.

The fertilization of ovulated eggs obviously depends on the meeting of germ cells in the oviduct at a time when sperm and eggs are viable (Bedu-Addo, 1993). There is evidence to support the fact that in the rat, fertilization rate rarely drops below 95 per cent (Nalbandov, 1976). Fertilization rates in all poloytoccous animals approach 100 per cent under favourable condition.

The results indicate that alcoholic extract of the fruits of *Jatropha curcas* causes significant reduction in implantation. The number of implantation sites counted for the dosed animals and the control animals significantly differed statistically. This establishes that the extract of fruits of *Jatropha curcas* affect implantation in the albino rats.

The effect on implantation could be brought about by the interference with the contractions of the uterus, or by the interference with the synchronization of charges in the endometrium or by the local interference with the basic hormonal sequence which systemically controls implantation. This brings about the cellular reactions in the endometrium necessary for the acceptance of the blasctocyst.

As argued earlier on, the extract does not contain the chemicals that could interfere with the basic hormonal sequence, which systemically controls implantation. The oestrogen secretion by the growing follicles was not inhibited by the extract. The

examination of sections of the uteri of the rats in the four treatment groups did not reveal any significant characteristic differences among them. The comparison of the sections of the uteri of the animals in the four treatment groups clearly showed that the extract did not affect the changes in the endometrium needed for implantation. The changes in the endometrium were therefore synchronized with the changes in the blastocyst necessary for implantation.

The possibly, the cause of the reduction in implantation in the rats could be due to the interference in the contraction of the uterus by the extract. Anto, in 1991 demonstrated that a crude aqueous extract of the young roots of *Jatropha curcas* caused the contraction of the guinea pig ileum (smooth muscle) in a dose-related manner as the conventional against, carbochol, histamine and nicotine. He also demonstrated that, the crude extract of the young roots of *Jatropha curcas* contracted isolated rat uterus in a dose related manner. The interference in the uterine contraction prevented the fertilized ova from implanting themselves to the uterus and hence the extract prevented implantation of ova.

The recorded results strongly suggest that alcoholic extract of *Jatropha curcas* brought about dose dependent ova wastage or foetal resorption in the albino. The administration of the extract started from day 6 of pregnancy, by which time the developing embryos had fully implanted themselves to the uterine walls of the animals.

The result revealed that the highest foetal resorption or abortion occurred in the animals which were given the highest dosage of the extract (68%) and the least ova wastage was recorded for the control animals (5%). This result endorses that work of

Goonasekara *et al.*, (1995) who demonstrated that extracts of fruits of *Jatropha curcas* interrupted pregnancy at an early stage of pregnancy after implantation.

As to the interference effect in uterine contraction by the extracted the cause of this abortifacient property of the extract of *Jatropha curcas* could be due to the presence of certain cytotoxic agents. The constituents of *Jatropha curcas* include Jatrophone and four novel diterpenes, curcusones A – D. Jatrophone has been demonstrated to be a cytotoxic agent and the novel diterpenes have also exhibited cytotoxic activity in KB cells culture (Chomnong, 1990). Cytotoxic agents can disrupt pregnancy possibly by interfering with mitotic divisions of the foetus. (Goonasekara, *et al.*, 1995.) The constituents in *Jatropha curcas* might cause foetal resorption by interrupting uterine contraction and mitosis in the developing foetus, and causing defected embryos to be aborted.

An observation made throughout the investigation was that the administration of the extract was causing decrease in weight of the female rats. There were significantly differences in reduction in weight of rat between the dosed animals and the control animals. The findings of this study was in agreement with Mameesh *et al.*, (1963) and Goonasekara *et al.*, (1995) who similarly recorded losses in weight the rats given *Jatropha curcas* extract.

These results suggest that the alcoholic extract of *Jatropha curcas* used might have interfered with protein metabolism and for that matter growth of the dams. The feeding of the rats could have also been affected by the extract.

Anto, (1991) reported of the presence of two phytosterols, a ptiytosterolin (a glucoside), large amounts of sucrose and resinous matter, having nauseating, purging and griping

effects present in *Jatropha curcas*. According to Afriyie (1994) increased nutrition is a pre-requisite of growth and reproduction. The ill effect of these constituents affected the feeding of these rats and caused reduction in weight.

There were no significant differences in the mortality rates among animals given the different concentrations of the extract. The occurrence of deaths in few of the female rats in the experiment could not therefore be attributed to the effect of the extract of the *Jatropha curcas*. It is normal for two out of thirty-two experimental animals to died by natural causes.

According to Irvine (1961), the fruits of *Jatropha curcas* have a poison, a resin (crotin resin) and this causes redness and pustular eruptions of the skin. These were not however observed in this work. Adam (1974) studied the effect of different dietary concentrations of *Jatropha curcas* seed on mortality and clinical response.

A high mortality was found in mice given diets of 50 and 40% *Jatropha* seed. The mortality was lower in those fed the seeds at concentrations of 20,10.and 5% of the feed. One percent of feed being *Jatropha curcas* did not cause death. According to Irvine (1961), there is also the presence of a toxic principle in *Jatropha curcas* which is resino-lipid in character. According to Oliver-Bever (1986), the seeds of *Jatropha curcas* contain a mucilage and a toxalbumin "curcin" with seven protein groups. The seed oil is a drastic purgative while highly irritant phorbol derivatives have also been isolated from the plant.

CHAPTER SIX

CONCLUSIONS AND SUGGESTIONS

6.1 CONCLUSION

There is substantial folkloric background information that a lot of herbs including *Jatropha curcas* are used for safe abortion in humans. There is also the speculation and reported findings that *Jatropha curcas* may be efficient as an abortifacient herb. It is against the above background information that this study was carried on. The study was conducted to verify the effects of an alcoholic extract of *Jatropha curcas* on some of the reproductive processes of the female albino rats, so that the information may be elucidated to humans.

Form the results obtained the following conclusions can be drawn for this project work. Within experimental errors, this project work concluded that the alcoholic extract of *Jatropha curcas* at dose levels between 2.5 and 1.5g per 1 kg body weight of rat.

- i. had no effect on the length of oestrous cycle, the development of the ova and ovulation in female albino rats.
- ii. had no effect on conception or pregnancy in the female albino rat
- iii. reduced implantation significantly in the female albino rat.
- iv. had significant effect on foetal loss or resorption or ova wastage the female rat.

In summary it can be concluded that alcoholic extract of the fruits of *Jatropha curcas* causes abortion in the female albino rat, and it is therefore an abortifacient in rats. *Jatropha curcas* causes abortion by reducing implantation and also interfering with the mitotic division of the developing fetuses.

Although the albino rats may respond to almost all potential drugs in a way which is markedly similar to other animals (Baker, *et al.*, 1977), this study does not conclude that alcoholic extract of the fruits of *Jatropha curcas* causes abortion in human beings. Nevertheless, this result points towards the possibility of *Jatropha curcas* being abortifacient in humans.

6.2 SUGGESTIONS

The time of the investigation did not permit investigation into the teratogenic effect of the extract of fruits (including seeds) of *Jatropha curcas*. For this reason, dimensions such as length and weights of the foetuses removed were not taken. The teratogenic effect of the alcoholic extract of fruits of *Jatropha curcas* could be investigated.

More detailed work could also be done to determine the mode of action of the abortifacient property of *Jatropha curcas*.

Furthermore, works should be done using different solvents such as methanol and dichloromethane for the extraction from the fruit of *Jatropha curcas* for the investigation.

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APPENDICES

Appendix 1

Table Ap 1: Technique used to determine the length of oestrous cycle.

(proportions of cells in vaginal smears of albino rats)

ALBINO RATS	Type of Cells Observed in Vaginal Smear	TIME (DAYS)																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
		Number of the different type of cous																				
A	Glandular	7	8	2	3	2	6	8	1*	0	5	3	5	6	0	0	4	5	*	2	0	0
	Cornified	0	0	8	3	1	0	0	8	7	3	1	0	3	10	3	0	1	*	8	10	8
	Infiltration	3	2	0	4	7	4	2	1*	3	2	6	5	1	0	7	6	4	*	0	0	2
B	Glandular	0	1	2	7	5	0	3	6	6	8	6	8	5	0	0	5	6	5	0		
	Cornified.	0	0	1	0	5	10	0	0	1	2	0	1	5	10	8	5	1	5	10		
	LeucocyticmInfiltration	10	9	7	3	0	0	0	7	4	0	4	1	0	0	2	0	3	0	0		
C	Glandular	9	9	-	-	4	7	1	0	0	7	2	5	5	9	1	5	5	10	2	1	4
	Cornified	0	1	-	-	0	0	9	7	3	2	8	1	0	1	9	3	1	0	8	0	0
	Leucocytic Infiltration	1	0	-	-	6	3	0	3	7	1	0	4	5	0	0	2	4	0	0	9	6
D	Glandular	-	-	-	-	1	5	10	0	1	4	6	3	0	0	6	5*	1	0	1	6	
	Cornified	-	-	-	-	0	2	0	10	2	0	0	6	10	3	0	2*	9	10	1	4	
	Leucocytic Infiltration	-	-	-	-	9	3	0	0	7	6	4	1	0	7	4	3*	0	0	8	0	
		* - thin smear ☐ oestrous smear OESTROUS LENGTH = Time lapse between two consecutive Oestrous smears																				

Appendix 2

Table Ap 2A

length of oestrous cycle of rats during the administration of the alcoholic extract of *Jatropha curcas*. [The extract was given for 16 consecutive days while the length of the oestrous cycle was being determined]

Treatment group	Animal identification	Oestrous cycle length (days)	Average	Mean length of oestrous cycle (days)
Group 3 animal (T3) [2.5g of extracted per 1 kg body weight of rat]	R ₁ R ₂ R ₃ R ₄ R ₅ R ₆ R ₇ R ₈	4, 4, 4, 5, 5, 5, 5, 5, 5, 3, 4, 4, 5, - - 4, - - 2, 4, 4, 5, 3, 4	4.0 5.0 5.0 3.7 died died 3.3 4.0	Died
Group 2 animal (T2) [2.5 of extracted per 1 kg body weight of rat]	R ₁ R ₂ R ₃ R ₄ R ₅ R ₆ R ₇ R ₈	Died 4, 4, 4, 5, 5, 5, 2, 5, 5, 4, 5, 4, 5, 5, 5, 4, 4, 4, 4, 4, 5	Died * 4.0 5.0 4.0 4.3 5.0 4.0 4.3	Died
Group 1 animal (T1) [2.5 of extracted per 1 kg body weight of rat]	R ₁ R ₂ R ₃ R ₄ R ₅ R ₆ R ₇ R ₈	5, 5, 5, 4, 4, 4,, 5, 5, 5,, 7 - - - died 4, 4, 4, 5, 5, 5, 4, 4, 4, 3, 4, 5	5.0 4.0 5.0 Died 4.0 5.0 4.0 4.0	Died
Control animals (To) [distil water]	R ₁ R ₂ R ₃ R ₄ R ₅ R ₆ R ₇ R ₈	5, 4, 4, 5, 4, 4 5, 5, 5, 5, 5, 5, 4, 4, 4, 3, 5, 5 4, 4, 4 5, 4, 4	4.3 5.0 5.0 4.0 4.0 4.3 4.0 4.3	

Table Ap 2B

LSD (T) COMPARISON OF MEANS of average length of the oestrus cycle
BY TREATMENT

TREATMENT	MEAN	HOMOGENEOUS GROUPS
1.5gex _ kg	4.4286	1
distil wat	4.4000	1
2. 0gex kg	4.3714	1
2. 5gex kg	4. 1667	1

THERE ARE NO SIGNIFICANT PAIRWISE DIFFEERENCES AMONG THE MEANS.

REJECTION LEVEL 0. 050

STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN
COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

Table Ap2C

STATISTIX FOR WINDOWS

ONE-WAY AOV FOR AVERAGE length of the oestrus cycle BY TREATMENT

SOURCE	DF	SS	MS	F	P
BETWEEN	3	0.26810	0.08937	0.33	0.8009
WITHIN	24	6.42190	0.26758		
TOTAL	27	6.69000			

	CHI-SQ	DF	P
BARTLETT'S TEST OF EQUAL VARIANCES	2.04	3	0.5636

COCHRAN'S Q	0.4290
LARGEST VAR / SMALLEST VAR	3.1284

COMPONENT OF VARIANCE FOR BETWEEN GROUPS	-0.02555
EFFECTIVE CELL SIZE	7.0

TREATMENT	MEAN	SAMPLE SIZE	GROUP STD DEV
distil H ₂ O	4.4000	8	0.3928
1.5gex _ kg	4.4286	7	0.5345
2.0gex _ kg	4.3714	7	0.4499
2.5gex _ kg	4.1667	6	0.6947
TOTAL	4.3500	28	0.5173
CASES INCLUDED	28	MISSING CASES	4

Appendix 3

Table Ap 3A.

Number of corpora lutea in the animals of the different treatment groups. (Animals were dosed for 16 consecutive days, then their ovaries were observed and the number of corpora lutea counted. The number of corpora lutea represent the number of eggs (ova) ovulated).

Treatment group (dosed)	Animal identification	Number of corpora lutea for individual animals
T3 (2.5g /kg)	R ₁	7
	R ₂	12
	R ₃	13
	R ₄	14
	R ₅	died
	R ₆	died
	R ₇	13
	R ₈	6
T2 (2.0g /kg)	R ₁	Died •
	R ₂	10
	R ₃	6
	R ₄	14 •
	R ₅	13
	R ₆	12
	R ₇	13
	R ₈	11
T1 (1.5g /kg)	R ₁	12
	R ₂	10
	R ₃	10
	R ₄	died •
	R ₅	9
	R ₆	8
	R ₇	12
	R ₈	13
To (distill water)	R ₁	13
	R ₂	5
	R ₃	9
	R ₄	14
	R ₅	14
	R ₆	11
	R ₇	5
	R ₈	11

Table Ap 3B

LSD (T) COMPARISON OF MEANS OF NUMBER OF COPORA LUTEA BY TREATMENT

TREATMENT	MEAN	HOMOGENEOUS GROUPS
2.0g / kg	11.286	1
2.5g / kg	10.833	1
1.5g / kg	10.571	1
distil H2O	10.250	1

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

REJECTION LEVEL 0.050
STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

Table Ap 3C

ONE-WAY AOV FOR NUMBER of copora lutea BY TREATMENT

SOURCE	DF	SS	MS	F	P
BETWEEN	3	4.23810	1.41270	0.16	0.9239
WITHIN	24	215.476	8.97817		
TOTAL	27	219.714			

BARTLETT'S TEST OF EQUAL VARIANCES	CHI-SQ	DF	P
	2.98	3	0.3946

COCHRAN'S Q	0.3747
LARGEST VAR / SMALLEST VAR	4.0652

COMPONENT OF VARIANCE FOR BETWEEN GROUPS	-1.08447
EFFECTIVE CELL SIZE	7.0

TREATMENT	MEAN	SAMPLE SIZE	GROUP STD DEV
distil H2O	10.250	8	3.6547
1.5g/kg	10.571	7	1.8127
2.0g/kg	11.286	7	2.6904
2.5g/kg	10.833	6	3.4303
TOTAL	10.714	28	2.9964
CASES INCLUDED	28	MISSING CASES	4

Appendix 4

Table Ap 4A

Number of female rats that showed positive mating (become pregnant) and number of implantation sites in the animals dosed up to the 5th day of pregnancy.

Dose Number (Dosage)	Individual Animal Identification	Presence or Absence of sperm in vaginal smear or plug (pregnancy)	Number implantation site Individual animals.
T3 (2...5g /kg)	R ₁₁	Pregnant	4
	R ₁₂	"	0
	R ₁₃	"	1
	R ₁₄	"	2
	R ₁₅	died	died
	R ₁₆	pregnant	7
	R ₁₇	"	1
	R ₁₈	"	3
T2 (2.5g /kg)	R ₁₁	Pregnant	3
	R ₁₂	"	4
	R ₁₃	"	2
	R ₁₄	"	6
	R ₁₅	"	7
	R ₁₆	"	1
	R ₁₇	"	7
	R ₁₈	"	9
T2 (1.0g /kg)	R ₁₁	Never pregnant	X
	R ₁₂	Never pregnant	X
	R ₁₃	Pregnant	7
	R ₁₄	"	6
	R ₁₅	"	5
	R ₁₆	"	3
	R ₁₇	"	8
	R ₁₈	"	2
To (distilled water)	R ₁₁	Pregnant	10
	R ₁₂	"	8
	R ₁₃	"	8
	R ₁₄	"	7
	R ₁₅	never Pregnant	X
	R ₁₆	pregnant	11
	R ₁₇	pregnant	12
	R ₁₈	"	9

Table Ap 4B (I)

LSD (T) COMPARISON OF MEANS for the presence of sperm smear (PREGNANCY)
BY TREATMENT

<u>TREATMENT</u>	<u>MEAN</u>	<u>HOMOGENEOUS GROUPS</u>
2. 0g / kg	1..0000	1
distilH2O	0.8750	1
2. 5g / kg	0.8750	1
1. 5g / kg	0.7500	1

THERE ARE NO SIGNIFICANT PARIWISE DIFFERENCES AMONG THE MEANS.

REJECTION LEVEL 0.050
CRITICAL VALUE FOR COMPARISON 0.3489
STANDARD ERROR FOR COMPARISON 0.1703

Table Ap 4B (II)

LSD (T) COMPARISON OF MEANS OF NUMBER of implantation sites of individual animals BY
TREATMENT

<u>TREATMENT</u>	<u>MEAN</u>	<u>HOMOGENEOUS GROUPS</u>
DistilH2O	9.2857	1
1.5g / kg	5. 1667	.. 1
2. 5g / kg	4. 8750	.. 1
2. 5g / kg	2. 5714	.. 1

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT
BECAUSE OF UNEQUAL SAMPLE SIZES.

Table Ap 4C (I)

ONE-WAY AOV FOR the presence of sperm smear (PREGNANY) BY TREATMENT

<u>SOURCE</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
BETWEEN	3	0.25000	0.08333	0.72	0.5496
WITHIN	28	3.25000	0.11607		
TOTAL	31	3.50000			

AT LEAST ONE GROUP VARIANCE IS NEAR ZERO;
VARIANCE-EQUALITY TESTS CANNOT BE COMPUTED.

COMPONENT OF VARIANCE FOR BETWEEN GROUPS -0.00409
EFFECTIVE CELL SIZE 8.0

<u>TREATMENT</u>	<u>MEAN</u>	<u>SAMPLE SIZE</u>	<u>GROUP STD DEV</u>
distil H ₂ O	0.8750	8	0.3536
1.5g/kg	0.7500	8	0.4629
2.0g/kg	1.0000	8	0.0000
2.5g/kg	0.8750	8	0.3536
TOTAL	0.8750	32	0.3407
CASES INCLUDED	32	MISSING CASES	0

Table Ap 4C (II)

ONE-WAY AOV FOR NUMBER of implantation sites of individual animals BY TREATMENT

SOURCE	DF	SS	MS	F	P
-----	----	-----	-----	-----	-----
BETWEEN	3	164.113	54.7044	9.74	0.0002
WITHIN	24	134.851	5.61880		
TOTAL	27	298.964			

	CHI-SQ	DF	P
-----	-----	-----	-----
BARTLETT'S TEST OF EQUAL VARIANCES	1.13	3	0.7709

COCHRAN'S Q	0.3553
LARGEST VAR / SMALLEST VAR	2.4210

COMPONENT OF VARIANCE FOR BETWEEN GROUPS	7.03616
EFFECTIVE CELL SIZE	7.0

TREATMENT	MEAN	SAMPLE SIZE	GROUP STD DEV
-----	-----	-----	-----
distil H ₂ O	9.2857	7	1.7995
1.5g/kg	5.1667	6	2.3166
2.0g/kg	4.8750	8	2.7999
2.5g/kg	2.5714	7	2.3705
TOTAL	5.4643	28	2.3704
CASES INCLUDED	28	MISSING CASES	4

Appendix 5

Table Ap 5A

Number of ova wasted, normal foetuses removed and corpora lutea in animals in the different treatment groups. [The administration of the extract started from day 6 of pregnancy, and continued for 10 consecutive days]

Treatment group	Animal identification	Number corpora lutea	Number of normal foetal removed	Number of ova wasted
T3 (2.5g /kg)	R ₂₁	8	0	8
	R ₂₂	11	0	7
	R ₂₃	3	0	3
	R ₂₄	died	died	died
	R ₂₅	12	2	10
	R ₂₆	14	3	11
	R ₂₇	5	3	2
	R ₂₈	12	8	4
T2 (2.0g /kg)	R ₂₁	10	5	5
	R ₂₂	13	3	10
	R ₂₃	4	0	4
	R ₂₄	9	5	4
	R ₂₅	5	0	5
	R ₂₆	7	0	7
	R ₂₇	13	9	4
	R ₂₈	12	6	6
T1 (1.5g /kg)	R ₂₁	4	2	2
	R ₂₂	9	4	5
	R ₂₃	6	2	4
	R ₂₄	14	11	3
	R ₂₅	12	6	6
	R ₂₆	11	11	0
	R ₂₇	10	9	1
	R ₂₈	12	11	1
To (distill water)	R ₂₁	13	12	1
	R ₂₂	14	13	1
	R ₂₃	8	8	0
	R ₂₄	8	8	0
	R ₂₅	9	9	0
	R ₂₆	8	6	2
	R ₂₇	9	9	0
	R ₂₈	7	7	0

Number of ova wasted = Number of corpora lutea - Number of Normal foetuses removed

Table Ap5B (I)

LSD (T) COMPARISON OF MEANS OF OVA wasted BY TREATMENTS

<u>TREATMENT</u>	<u>MEAN</u>	<u>HOMOGENEOUS GROUPS</u>
2.5g/kg	6.4286	1
2.0g/kg	5.8571	1
1.5g/kg	2.7500	..1
distilH2O	0.5000	..1

THERE ARE 2 GROUPS IN WHICH MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

REJECTION LEVEL 0.050

STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

Table AP 5B (II)

LSD (T) COMPARISON OF MEANS OF NORMAL FOETUS REMOVED BY TREATMENT

<u>TREATMENT</u>	<u>MEAN</u>	<u>HOMOGENEOUS GROUPS</u>
DistilH2O	9.0000	1
1.5g/kg	7.0000	1
2.0g/kg	3.5000	..1
2.5g/kg	2.2857	..1

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.052 REJECTION LEVEL 0.050

STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

Table Ap5C (I)

ONE-WAY AOV FOR NO. of ova wasted BY TREATMENT

<u>SOURCE</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
BETWEEN	3	173.795	57.9317	11.07	0.0001
WITHIN	26	136.071	5.23352		
TOTAL	29	309.867			

<u>BARTLETT'S TEST OF EQUAL VARIANCES</u>	<u>CHI-SQ</u>	<u>DF</u>	<u>P</u>
	11.63	3	0.0088

COCHRAN'S Q	0.5627
LARGEST VAR / SMALLEST VAR	21.500

COMPONENT OF VARIANCE FOR BETWEEN GROUPS	7.03686
EFFECTIVE CELL SIZE	7.5

SAMPLE GROUP

TREATMENT	MEAN	SIZE	STD DEV
distil H ₂ O	0.5000	8	0.7559
1.5g/kg	2.7500	8	2.1213
2.0g/kg	5.8571	7	2.1157
2.5g/kg	6.4286	7	3.5051
TOTAL	3.7333	30	2.2877
CASES INCLUDED 30 MISSING CASES 2			

Table Ap 5C (II)

ONE-WAY AOV FOR NORMAL FOETUSES REMOVED BY TREATMENT

SOURCE	DF	SS	MS	F	P
BETWEEN	3	220.249	73.4163	7.09	0.0012
WITHIN	27	279.429	10.3492		
TOTAL	30	499.677			

	CHI-SQ	DF	P
BARTLETT'S TEST OF EQUAL VARIANCES	1.85	3	0.6052

COCHRAN'S Q	0.3893
LARGEST VAR / SMALLEST VAR	2.8000

COMPONENT OF VARIANCE FOR BETWEEN GROUPS	8.14616
EFFECTIVE CELL SIZE	7.7

TREATMENT	MEAN	SAMPLE SIZE	GROUP STD DEV
distil H ₂ O	9.0000	8	2.3905
1.5g/kg	7.0000	8	4.0000
2.0g/kg	3.5000	8	3.3381
2.5g/kg	2.2857	7	2.8702
TOTAL	5.5484	31	3.2170
CASES INCLUDED 31 MISSING CASES 1			

Table Ap 6A(I)

Weight of the female rats during the experimental period.

(The animals were given the different dosages of the extract of *Jatropha curcas* for 16 consecutive days and their weights were taken once in every 4 days).

Treatment Group (Dose)	Individual Animal Identification	Weight (g)				
		Day 0	Day 4	Day 8	Day 12	Day 16
T3 (2.5g /kg)	R ₁	210	200	190	190	180
	R ₂	230	230	210	200	200
	R ₃	250	145	245	240	230
	R ₄	165	165	160	150	140
	R ₅	160	dead	-	-	-
	R ₆	200	210	200	190	180
	R ₇	150	140	140	130	120
	R ₈	150	150	150	140	130
T2 (2.0g /kg)	R ₁	165	150	Dead	Dead	Dead
	R ₂	190	190	190	180	175
	R ₃	170	170	170	160	150
	R ₄	200	210	210	200	200
	R ₅	190	180	185	170	170
	R ₆	210	210	200	190	190
	R ₇	240	240	235	230	230
	R ₈	160	160	150	140	150
T1 (1.5g /kg)	R ₁	210	210	200	200	200
	R ₂	180	170	170	170	170
	R ₃	170	175	180	180	180
	R ₄	180	170	died	died	died
	R ₅	180	180	190	190	180
	R ₆	170	160	150	150	150
	R ₇	220	220	230	230	230
	R ₈	200	210	215	215	215
To (Distilled water) control	R ₁	190	190	200	200	210
	R ₂	175	180	180	185	185
	R ₃	200	200	200	210	220
	R ₄	250	250	240	240	240
	R ₅	220	220	230	230	220
	R ₆	215	215	220	220	220
	R ₇	170	170	175	175	175
	R ₈	150	160	160	155	150

Table Ap 6A(II)

Increase (Decrease) in weight over 16 days of administration of the extract of *Jatropha curcas*

Treatment Group (Dose)	Individual Animal Identification	Increase in weight (g)				
		Day 0	Day 4	Day 8	Day 12	Day 16
T3 (2.5g /kg)	R ₁	0	-10	20	-20	-30
	R ₂	0	0	20	-30	-30
	R ₃	0	-5	-5	-10	-20
	R ₄	0	0	-5	-15	-20
	R ₅	0	Died	-	-	-
	R ₆	0	+10	0	-10	-20
	R ₇	0	-10	-10	-20	-30
	R ₈	0	0	0	-10	-20
T2 (2.0g /kg)	R ₁	0	-15	Dead	-	Died
	R ₂	0	0	0	-10	-15
	R ₃	0	0	0	-10	-20
	R ₄	0	0	+10	-10	0
	R ₅	0	+10	-5	0	-20
	R ₆	0	-10	-20	-20	-20
	R ₇	0	0	0	+10	+10
	R ₈	0	0	-10	-20	-10
T1 (1.5g /kg)	R ₁	0	0	0	-10	-10
	R ₂	0	-10	-10	-10	-10
	R ₃	0	+5	+10	+10	+10
	R ₄	0	-10	died	died	died
	R ₅	0	0	0	+10	0
	R ₆	0	-10	-20	20	-20
	R ₇	0	0	0	+10	+10
	R ₈	0	+10	+10	+15	+15
To (Distilled water) control	R ₁	0	0	+10	+10	+20
	R ₂	0	+5	+5	+10	+10
	R ₃	0	0	0	+10	+20
	R ₄	0	0	-10	-10	-10
	R ₅	0	0	+10	+10	0
	R ₆	0	0	+5	+5	+5
	R ₇	0	0	+5	+5	+5
	R ₈	0	+10	+10	+5	0

LSD (T) COMPARISON OF MEANS OF DAY_4 BY TREATMENT

TREATMENT	MEAN	HOMOGENEOUS GROUPS
distil	1.8750	I
1.5g/kg	-1.8750	I
2.0g/kg	-1.8750	I
2.5g/kg	-2.5298	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 7.1317
 STANDARD ERROR FOR COMPARISON 3.4189

ERROR TERM USED: REP*TREATMENT, 20 DF

LSD (T) COMPARISON OF MEANS OF DAY_8 BY TREATMENT

TREATMENT	MEAN	HOMOGENEOUS GROUPS
distil	4.3750	I
1.5g/kg	0.0281	I I
2.0g/kg	-1.4923	I I
2.5g/kg	-8.4719	.. I

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE
 NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 8.9657
 STANDARD ERROR FOR COMPARISON 4.2495

ERROR TERM USED: REP*TREATMENT, 17 DF

LSD (T) COMPARISON OF MEANS OF DAY_12 BY TREATMENT

TREATMENT	MEAN	HOMOGENEOUS GROUPS
distil	5.6250	I
1.5g/kg	1.4031	I I
2.0g/kg	-7.7423	.. I I
2.5g/kg	-17.347	 I

THERE ARE 3 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

REJECTION LEVEL 0.050
CRITICAL VALUE FOR COMPARISON 9.7532
STANDARD ERROR FOR COMPARISON 4.6228

ERROR TERM USED: REP*TREATMENT, 17 DF

LSD (T) COMPARISON OF MEANS OF DAY_16 BY TREATMENT

TREATMENT	MEAN	HOMOGENEOUS GROUPS
distil	6.2500	I
1.5g/kg	-3.6071	I I
2.0g/kg	-9.6429	.. I
2.5g/kg	-25.857	 I

THERE ARE 3 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

REJECTION LEVEL 0.050
CRITICAL VALUE FOR COMPARISON 11.808
STANDARD ERROR FOR COMPARISON 5.5967

ERROR TERM USED: REP*TREATMENT, 17 DF

STATISTIX FOR WINDOWS

ANALYSIS OF VARIANCE TABLE FOR DAY_4

SOURCE	DF	SS	MS	F	P
REP (A)	7	456.746	65.2494	1.40	0.2612
TREATMENT (B)	3	96.7687	32.2562	0.69	0.5688
ERROR	20	935.119	46.7560		
TOTAL	30	1488.63			

CASES INCLUDED 31 MISSING CASES 1

ANALYSIS OF VARIANCE TABLE FOR DAY_8

SOURCE	DF	SS	MS	F	P
REP (A)	7	492.097	70.2996	0.97	0.4810
TREATMENT (B)	3	683.284	227.761	3.15	0.0520
ERROR	17	1227.96	72.2329		
TOTAL	27	2403.34			

CASES INCLUDED 28 MISSING CASES 4

ANALYSIS OF VARIANCE TABLE FOR DAY_12

SOURCE	DF	SS	MS	F	P
REP (A)	7	1180.25	168.607	1.97	0.1197
TREATMENT (B)	3	2503.34	834.447	9.76	0.0006
ERROR	17	1453.17	85.4804		
TOTAL	27	5136.76			

CASES INCLUDED 28 MISSING CASES 4

ANALYSIS OF VARIANCE TABLE FOR DAY_16

SOURCE	DF	SS	MS	F	P
REP (A)	7	1120.35	160.050	1.28	0.3184
TREATMENT (B)	3	4350.02	1450.01	11.57	0.0002
ERROR	17	2130.00	125.294		
TOTAL	27	7600.37			

CASES INCLUDED 28 MISSING CASES 4