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The present study describes the novel use of a traditional plant product, neem (Azadirachta indica) oil, for a long term and reversible blocking of fertility after a single intrauterine application. A single administration of 100 ul of the oil per uterine horn is sufficient to block fertility for a minimum period of 100 days or more in rodents. The effect is specific for neem oil since peanut oil given by the same route does not have any effect on fertility. At lower concentrations of neem oil, the antifertility effect of neem oil is seen for shorter periods and the animals revert back to fertility much earlier. Since the method employs a single pre-coital intrauterine application of the oil, it is distinct from the previous studies, both in terms of the route employed and the mechanism involved.

Neem oil has been traditionally used as an abortificient agent by ancient Indian physicians. More recent investigations have also focussed attention on the antifertility effect following post coital application of the oil (Sinha et al, 1984b; Lal et al, 1986; Tewari et al, 1986) and its spermicidal effect (Sinha, 1984a). The present study is however distinct from the earlier work in many aspects. The spermicidal action of the oil described in earlier studies required repeated intravaginal application before each coitus. The post-coital application also demanded a 3-10 day regimen. In the previous investigations the intravaginal or oral route had been used for administration of the oil. The efficacy was partial ranging from 25-100% depending on the dose of the oil and the time of its administration, in the case of intravaginal route and even lesser in the oral route (20-40%). In one report (Sinha et al, 1984b), where restoration of fertility has been studied, 30% animals recovered fertility following the withdrawal of oil application for 30 days, hence the block is effective only for 30 days. In contrast, the novel manner in
which the treatment was given in the present study led to a block in fertility for a
minimum period of 100 days.

The present study also shows that the intrauterine application of neem oil
does not interfere with follicular development, ovulation, reproductive cyclicity
and libido. This was evidenced by ovarian histology and vaginal smears of the
treated animals, which were put on continuous mating with males of proven
fertility following the treatment. The presence of corpus luteum and growing
follicles in the ovaries of treated animals demonstrated the normal morphology of
the ovaries. Its functional normalcy was confirmed by the progesterone profiles
seen in these animals, which indicated normal ovulatory cycles. The treated
animals had regular estrus cycles and showed repeated sperm positivity. Another
interesting feature noted in these studies which indicated the normal cyclicity of
the treated animals was the fact that they became pseudopregnant following each
mating - a feature which is well documented in rodents, where sterile mating with
vasectomized males induces pseudopregnancy in females (Rowlands and Weir,
1984).

No pathological changes are noted in the uterus of the female rats following
application of the oil. This was evident by the normal morphology of the uterus
following administration of the oil.

One of the possible mechanisms by which neem oil could cause infertility
maybe by interfering with the physiological responses of the uterus to the ovarian
hormones, leading to a change in the uterine receptivity to the embryos and causing
their degeneration. This study shows that intrauterine instillation of neem oil in
rats does not change the receptivity of the uterus. Administration of exogenous
estradiol increased the uterine weight significantly in neem oil treated,
ovariectomized rats. This increase was similar to the gain in weight demonstrated by peanut oil treated control group. Peanut oil was taken as control since it was already shown that intrauterine administration of peanut oil did not affect the fertility of female rats. The histological features of the uterus of neem oil administered females also showed stimulation in response to estradiol. The induction of decidualization in the uterus of neem oil treated animals following mechanical stimulation on Day 5 post-coitum confirmed the progesterone priming and the hormonal conditioning of the uterus. It is known that implantation in rodents follows a precise time course of hormonal conditioning. A progesterone priming for about 48 hours which then allows a few hours later, estrogen to induce a short phase of endometrial receptivity. Any change in this normal pattern of hormonal conditioning can alter the uterine receptivity, interfering with implantation. The results of this study show that the antifertility effect of neem oil is not due to a change in the receptivity of endometrium to the embryo.

The possible antihormonal action of neem oil has been a subject of speculation in earlier studies done by other investigators. Riar et al (1986) administered neem oil intravaginally on different days post-coitum and ligated the uterine horn to prevent the direct action of the oil. They suggested that the block in fertility could be due to absorption of active components of neem oil through the vaginal mucosa into the circulation, exerting an anti-estrogenic effect. The post implantation application of the oil was speculated to cause a fall in the peripheral levels of progesterone. This possibility was ruled out by studies done by Prakash et al (1988). They reported that injection of 0.3 ml neem oil into female rats by the subcutaneous route did not have any estrogenic, anti estrogenic or progestational activity per se. Nor did it interfere with the action of progesterone. The result of the present study are in concurrence with these findings. Prakash et al (1988), in the same study, saw a severe damage to the uterine endometrium
which could be the cause of infertility. The present study has clearly shown that intrauterine administration of neem oil does not have any effect on the normal functional morphology of the reproductive organs. Direct histological examination of the ovaries and the uterus of the animals administered neem oil through the intrauterine route proved that the uterine and ovarian histoarchitecture remained intact. Other groups (Riar et al, 1988), have also reported the lack of any histopathological effects on uterus, cervix and ovaries of rats treated intravaginally with neem oil.

The present study demonstrates that the antifertility effect seen following intrauterine administration of neem oil is localized in action. The animals receiving neem oil in one uterine horn had no fetuses in that horn; nor was any site of implantation or fetal resorption noted. The control horn receiving peanut oil had normal implantation sites and growing embryos. The block in fertility is at the pre-implantation stage. Embryos flushed out on different days following coitus (Days 3-5) after neem oil administration, were found to be undergoing degeneration, while the control horn had normally developing embryos. The presence of degenerated embryos corresponded with the phenomenon of marked leukocytic infiltration into the uterine epithelium around the same time. Maximum infiltration was observed around days 3-5 post coitum which corresponds to the pre-implantation period in rodents. The cells consisted mainly of macrophages, lymphocytes and polymorphonuclear cells. The control side on the other hand had no signs of leukocytic infiltration in the corresponding period. The phenomenon of leukocytosis is however, transient in nature since no signs of infiltration were noticeable 8-10 days after mating. Previous studies by Anderson and Alexander (1979) have shown that non specific leukocytosis in the uterus affects fertility in rats. In their study they have used an inert agent like glycogen as the chemotactic agent for attracting PMNs. The anti-implantation effect of IUDs is also due to the
induction of a local inflammatory response leading to leukocytic infiltration into the uterus. Increased number of leukocytes in the peritoneal cavity has also been implicated in causing infertility among endometriosis patients (Haney et al, 1981; Halme et al, 1982; Chacho et al, 1986; Hill et al, 1989a).

The presence of MHC class-II positive cells in normal rat uterus has been demonstrated by Head (1987). It has been shown earlier that although Class-II histocompatibility antigens are expressed on a variety of cell types, they are predominately present on populations of cells having antigen presenting activity including macrophages, dendritic cells and Langerhans cells (Steinman and Nussenzweig, 1980). Following administration of neem oil the antigen presenting capacity of the uterine epithelium is considerably enhanced. This is indicated by the increased presence of Ia antigen on the epithelial cells of the neem oil treated uterine horn as compared to the control side. Recent studies on immunomodulatory effects of neem oil have also demonstrated enhanced levels of MHC class-II on peritoneal macrophages, following intraperitoneal neem oil administration (Upadhyay et al, 1992). It is therefore possible that the neem oil administered in the uterus enhances the antigen presenting ability of the uterine epithelium. On sperm challenge, following mating, instead of a facilitatory immune response as is seen following normal mating there is a leukocytic infiltration, consequently leading to degeneration of embryo and their inability to implant.

The involvement of the local immune cell population in causing the embryo degeneration is further indicated by the results obtained by culturing the local draining lymph node cells. There was significantly enhanced proliferation of the cells in the lymph nodes draining the uterus, following mating, in neem oil treated rats compared to that seen in the control sperm positive female rats. The supernatant collected from these cells had embryocidal activity as seen by the
effect on mouse embryos cultured in these supernatants. Significant degeneration of embryos was seen in supernatants from lymph node cells of day 3-5 post coitum, which corresponds to the pre-implantation period. The activity seen in the supernatant was heat labile and abrogated when digested with trypsin. Hence it appears to be due to a protein factor.

It is known that the draining lymph nodes are a good index of the local immune response in the uterus (Beer and Billingham, 1976). They have reported that uteri of hybrid females inoculated with suspension of lymph node cells from parental strain donors underwent graft-versus-host (GVH) reaction. This was accompanied by rapid enlargement and enhanced cellularity in the para-aortic nodes. The soluble products of activated macrophages and lymphocytes, the monokines and the lymphokines, have been shown to inhibit sperm motility and embryo development (Hill et al., 1987a,b). Naz and Mehta (1989) have shown that presensitized lymphocytes can secrete soluble mediators that activate macrophages and significantly inhibit sperm motility and embryonic development. The observed antifertility of neem oil maybe mediated by the products of activated macrophages and lymphocytes.

From this study it becomes clear that the antifertility effect of neem oil is mediated by induction of non specific local cellular immune response. Two possibilities exist as to what the exact mechanism of action could be. Firstly it is possible that the neem oil activates the local population of uterine leukocytes and enhances antigen presenting ability of uterine epithelium. Subsequent exposures to allogenic sperm or pre-implantation embryo, after mating initiate a cellular immune response within the uterus, leading to degeneration of embryos and their inability to implant. In fact, the local cell mediated immunity to sperm or embryo in the female reproductive tract is now recognized as one of the causes of
immunologic infertility in humans (Anderson and Hill, 1988). There are also reports where experimental induction of local immune response specific to sperm or sperm antigens leads to a decrease in fertility (Anderson and Alexander, 1979; Naz and Mehta, 1989). Moreover non-specific immune response to unrelated substances or against microbial infections have also been implicated in infertility. The IUDs, based on the same principle, induce a local inflammatory reaction. Large population of immune cells, including macrophages, lymphocytes and neutrophils have been recovered from IUDs after long usage. The T lymphocytes scrapped from IUDs showed a significant in vitro proliferative response to mitogens (Ogra, 1981).

An alternative possibility is that the intrauterine administration of neem oil might interfere with or block the local immunosuppressive mechanisms that provide protection to spermatozoa or the pre-implantation embryo (Clark and Slapsys 1985; Hoversland and Beaman 1990), leading to the rejection of the embryo. It is known that during the normal reproductive process there are immunoregulatory mechanisms within the female reproductive tract which prevent the local immune mechanisms from reacting against the presence of allogenic sperm or embryo.

Further support to the present study is lent by the reported immunomodulatory activities seen in various extracts and components isolated from neem. The polysaccharide present in the aqueous extract of the stem bark has been shown to have antitumor, -interferon inducing activity (Fujiwara et al, 1982, Teruma Corp. patent, 1985). Recently it has been reported that neem oil activates the phagocytic activity of peritoneal macrophages and induces production of -interferon by T cells. It acts as a non specific immunostimulant,
activating selectively the cell mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge (Upadhyay et al, 1992). From the present study it can be concluded that the intrauterine application of neem oil leads to a long term block in fertility. The block is at the pre-implantation stage and is due to the degeneration of embryos caused by the non specific activation of local immune cell population. Neem oil possibly activates the macrophages and other cells of the uterine endometrium. Subsequently, during mating, when the sperms are introduced into the uterus, instead of eliciting a suppressive reaction as under normal circumstances, they evoke a rejection type of immune response. This is reflected by transient leukocytic infiltration seen in the uterus and proliferation of lymphocytes seen in the draining lymph nodes. The leukocytes and possibly their secreted products lead to the degeneration of the embryo before it can implant.

The absence of any systemic toxic effect following the administration of oil is put in evidence by the effect seen following unilateral administration of neem oil. Block in pregnancy was seen only in the neem oil treated horn, while normal embryos were present on the contralateral horn. The administration of oil did not have toxic effect on embryo development on the contralateral uterine horn. The unilaterally treated animals, as well as some of those on long term fertility studies delivered normal pups ruling out the possibility of any long lasting teratogenic effect.

The reversibility of the phenomenon may by explained by the fact that the effect lasts only for as long as the activation of the macrophages is there. The normal life of tissue macrophages is 3-6 months in rats which is the period for
which the block in fertility is seen. The exact mechanism behind the activation of cells and the time duration of the phenomenon is still a matter of speculation.

The present study, for the first time proposes a novel use of a traditional plant product, neem oil, for a long term and reversible method of contraception following a single intrauterine administration with 100% efficacy. The method is safe, free from any systemic side effect and requires a single application. Moreover, it does not affect the hormonal cyclicity.