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Influence of Flaxseed (*Linum usitatissimum*) on Female Reproduction

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ABSTRACT

This review describes the chemical composition of flaxseed (*Linum usitatissimum*) and its general health effects, as well as the currently available knowledge concerning its action on the female reproductive state, functions on the ovary and ovarian cells and reproductive hormones, as well as possible constituents and extra- and intracellular mediators mediating its effects on female reproductive processes. Flaxseed contains a number of biologically active molecules, which, acting through multiple signalling pathways, can determine numerous physiological, protective and therapeutic effects of flaxseed. The available publications demonstrate the action of flaxseed and its constituents on the female reproductive system – ovarian growth, follicle development, the resulting puberty and reproductive cycles, ovarian cell proliferation and apoptosis, oo- and embryogenesis, hormonal regulators of reproductive processes and their dysfunctions. These effects can be determined by flaxseed lignans, alpha-linolenic acid and their products. Their actions can be mediated by changes in general metabolism, metabolic and reproductive hormones, their binding proteins, receptors and several intracellular signalling pathways, including protein kinases, transcription factors regulating cell proliferation, apoptosis, angiogenesis and malignant transformation. Flaxseed and its active molecules are found potentially useful for improving farm animal reproductive efficiency and treatment of polycystic ovarian syndrome and ovarian cancer.

Introduction

Flaxseed (*Linum usitatissimum* L, family *Lineaceae*) contains a number of biologically active substances, which define its nutritional, physiological and therapeutic value. The physiological and medicinal properties of this plant have been described in a number of reviews [1–4]. On the other hand, no special reviews of available information concerning flaxseed action on reproductive processes have been published yet. The present publication is the first attempting to review the current available knowledge concerning the action of flaxseed on the healthy female reproductive system, as well as of the data concerning its ability to prevent and to treat reproductive disorders.

The search for literature was performed in agreement with the PRISMA-ScR criteria [5]. Related articles were searched for in the PubMed, Web of Science and SCOPUS databases from 1995 to 2021. In cases of repeated or conflicting information or references, more recent sources have been preferred. Words used to search were “flaxseed” or “linseed”, alone or in combination with “health”, “metabolism”, “ovarian”, “oocyte”, “fertility” and “mechanisms”. Both relevant experimental papers and the reviews were evaluated. A total of 1321 research papers and reviews were inspected, and 604 were considered as useful and suitable for analysis.

Provenance and Properties

Flaxseed or linseed (*Linum usitatissimum*) is a plant from the Linaceae family. It has been cultivated in Egypt and Samaria since approximately 10 000 years ago and in China and India for 5000 years. [6]. Cultivated flaxseed can be divided from the economic standpoint: flax for fiber/linen, for oil (as a component of human and animal food and of fuel), or for oil and linen. In addition, in recent decades, flaxseed started being used for phytoremediation of soils contaminated by heavy metals [6].

Seed of oil flax contains 30–45% fat (drying oil whose main components are polyunsaturated acids – linoleic and linolenic acids), 20–30% protein, 8% water and up to 28% soluble and insoluble fibre. In the seed coat, the soluble fibre creates gel, which can swell to multiple times its size. The seed contains 1% cyanide glycosides, of which the best-known are lignans, as well as a high content of vitamin E [1–3].

Flaxseed is the richest source of the lignan secoisolariciresinol diglucoside – a compound found in the outer layers of flaxseed. In rumen, it is conversed to the enterolignans, enterodiol and enterolactone, whilst the important role of rumen microbiota in such a conversion has been demonstrated [7].

The available data concerning bioavailability and metabolic conversion of flaxseed oils are contradictory: several animal studies did not show an increase in plasma omega-3 (*n*-3) fatty acids level after flaxseed consumption [8]. Dietary flaxseed did not affect the concentration of its components – lignan enterolactone [9], *β*-hydroxybutyrate and nonesterified fatty acids [10] – in cow plasma. In rats, dietary flaxseed oil did not increase but even reduced blood cholesterol, triglycerides and low-density lipoproteins [11]. On the other hand, feeding gilts with flaxseed increased levels of alpha-linolenic acid, timodonic and cervonic acids and decreased concentrations of myristic, palmitic and palmitoleic acids in gilt plasma [12]. Feeding cows with flaxseed alpha-linolenic acid resulted in accumulation of this acid and its metabolites in cow milk [7, 13], plasma [9], ovarian follicular fluid and oocyte-cumulus complexes [9, 14, 15]. Metabolites of lignans, enterolactones can also be accumulated in milk and together with milk can enter animal and human organisms [7]. Therefore, the dietary flaxseed molecules can enter general circulation and accumulate in female reproductive organs.

Physiological Action

The performed *in vitro*, animal and clinical studies [1–4, 7, 16–20] report a number of physiological and therapeutic effects of flaxseed. Antioxidant and antithrombotic effects enable the reduction in the blood level of overall cholesterol, LDL cholesterol and triacylglycerols, to prevent metabolic (type II diabetes, obesity) and cardiovascular (hypertension, arteriosclerosis and ischemia) diseases and to improve memory. Flaxseed can prevent or treat tumour development and chemical intoxication, including intoxication induced by neurotoxic warfare organophosphate nerve agents. Flaxseed is a stimulator of immunity, but it has an anti-inflammatory effect and can prevent autoimmune disorders (psoriasis, systemic lupus erythematosus, asthma, rheumatoid arthritis, etc.). Flaxseed can improve the health and hygiene of the colon and the maintenance of gastrointestinal microbiota. For the valuable properties of its gel, flaxseed is used as a treatment against constipation and to stimulate bowel activity, to treat stomach irritation and stomach ulcers and to prevent stomach cancer, as well as for bronchitis and inflammations of urinary tract [1–4, 6, 19, 20].

Flaxseed oil is applied externally in dermatology. It is very well-applicable in treatment of skin diseases and burns and as a regenerative cosmetic preparation for regular skin treatment. Isolated esters of fatty acids are applied in medicinal cosmetology as ingredients in regenerative preparations. Flaxseed constituents help maintain healthy hair and skin – reducing redness and flaking, treating acne and eczema. Flaxseed can serve as a substitute for flour containing gluten for those who suffer from celiac disease [3, 4, 6, 17].

Well-known are the positive effect of flaxseed on general and fat metabolism. Studies on rats showed that flaxseed meal decreased the levels of fatty acids in their blood and the size of their adipose cells but not the feed intake or body weight [21]. In humans, flaxseed consumption mitigated inflammation and insensitivity to glucose and insulin [16] and reduced digestibility of lipids [22]. On the other hand, clinical studies did not determine its effect on body weight and obesity in humans [16, 22].

Taken together, the available data demonstrate a positive effect of flaxseed on a wide plethora of physiological processes, dysfunctions and illnesses.

No adverse side-effects were determined in the consumption of flaxseed products at therapeutic doses. However, when large doses are ingested, possible intoxication by hydrogen cyanide, which the seeds release during milling, cannot be excluded. Long-term use of flaxseed at therapeutic doses is not recommended without medical supervision in these cases: intestinal blockage, acute appendicitis, pancreatitis, peritonitis and acute painful hernia. Pregnant women should opt for flaxseed oil rather than milled flaxseed – the oil does not contain lignans with phytoestrogenic effect, which could trigger complications in pregnancy. These possible dangers, however, have not been validated by experiments and flaxseed products can be considered safe. The U.S. Food and Drug Administration (FDA) considers flaxseed products safe for nursing women and the nutrition of children [23].

Mechanisms of Action

Flaxseed constituents responsible for its physiological effects

The comparison of physiological effects of flaxseed and its constituents listed above enabled the identification of the flaxseed molecules responsible for its physiological and therapeutic effects [1, 3, 4, 7, 16, 17, 20, 24]. The authors reported that cardiovascular diseases (arteriosclerosis and ischemic disease, hypertension, hypercholesterolemia) can be prevented by flaxseed alpha-linoleic fatty acid, lignans, phenolic acids, E vitamin and folic acid, which possess antioxidant, anticholesterol and antithrombotic properties. Flaxseed has anti-tumour properties that are due to the presence of alpha-linoleic fatty acid, lignans, gamma tocopherol (E vitamin), folic acid (B9 vitamin), magnesium, phenolic acid and flavonoids with anti-oxidative and anti-proliferative properties. The flaxseed alpha-linoleic acid, docosahexaenoic acid and 2-methoxyestradiol, a steroid metabolite up-regulated by flaxseed, suppress angiogenesis during tumour development. The anti-type II diabetic effect of flaxseed could be due to its alpha-linoleic acid, lignans, gamma tocopherol (E vitamin), lignans and fibre. Alpha-linoleic acid, lignans and gamma tocopherol (E vitamin) can be responsible also for the immune-stimulatory action of flaxseed. Alpha-linoleic fatty acid could be beneficious also for

the treatment of autoimmune disorders (psoriasis, systemic lupus erythematosus, asthma, rheumatoid arthritis, etc.). Flaxseed fibre, E vitamin, lignans, vitamins and minerals can promote gastrointestinal tract activity and the maintenance of gastrointestinal microbiota, which in turn is important for fat metabolism and immune processes. The positive effect of flaxseed on memory can be explained by the presence of omega-3 fatty acids. These acids and lignans also suppress inflammatory processes. The ability of flaxseed to bind and to prevent chemical toxins can be due to the presence of omega-3 fatty acids, fibre and lignans.

Mediators of flaxseed effects

The intracellular mediators of flaxseed action on some physiological processes and illnesses have been described in a series of special reviews [3, 18, 25, 26]. The anti-hypertensive, anti-atherosclerotic, anti-platelet aggregation and cardioprotective effects of flaxseed proteins and other molecules could be due to its ability to suppress inflammatory processes via down-regulation of pro-inflammatory transcription factor NF-κB [3]. The involvement of intracellular promoters of cell proliferation and apoptosis in control of these processes and cancerogenesis, which are changed under the influence of flaxseed molecules, is also documented. Flaxseed can promote vascular hyperemia of various organs via the promotion of the generation of vasodilatory nitric oxide. In addition, flaxseed α-linolenic acid can inhibit soluble epoxide hydrolase, which in turn can promote vasodilation and induce production of inflammatory and cytotoxic oxylipins, metabolites of n-3 polyunsaturated fatty acids [3]. Flaxseed enterolactones have high antioxidant properties (ability to neutralize reactive oxygen species directly or via up-regulation of antioxidant enzymes [7]). Due to the antioxidant [7] and antiestrogenic [27, 28] (see also below) properties, flaxseed and enterolactones can suppress the development of cancer in various organs, suppressing mutagenesis and the promoters of cell proliferation, survival, angiogenesis, inflammation and metastasis [20, 25, 26]. The neuroprotective action of flaxseed α-linolenic acid can be explained by the activation of endogenous neuroprotective and neurorestorative pathways in the brain via up-regulation of the transcription factor NF-κB, a brain-derived neurotrophic factor and its receptor [18].

There is evidence that flaxseed can exert its physiological actions not only through intracellular but also extracellular regulators – hormones, growth factors and cytokines. For example, flaxseed proteins could suppress inflammatory processes via the down-regulation of cytokines interleukines and tumour necrosis factors and to affect angiogenesis through changes in vascular endothelial growth factors [3]. Furthermore, flaxseed lignans possess high phytoestrogen properties. They could either up- or down-regulate oestrogen receptor alpha [27, 28] and decrease the expression of oestrogen receptor alpha [27] and increase production of 2-methoxyestradiol and to affect oestrogen metabolism [24]. These actions on steroid hormones production and reception could explain their ability to affect the development of oestrogen-dependent breast cancer or oestrogen-dependent glucose metabolism and cardiovascular risk in postmenopausal individuals [25, 28]. Finally, flaxseed can down-regulate the IGF (insulin-like growth factor)/insulin signalling pathway [27], which is involved in the control of metabolic, proliferative and reparative processes.

These examples illustrate the multiple molecules and pathways mediating the influence of flaxseed and its constituents on various targets and defining the curative effect of flaxseed on some disorders.

Effects on Female Reproductive Processes

Effect on ovarian and reproductive state

The experiments performed *in vivo* on laboratory and farm animals demonstrated that flaxseed and its constituents can affect ovarian growth, follicle development and the resulting puberty and reproductive cycles.

Experiments on rats demonstrated both the stimulatory and inhibitory actions of flaxseed on their reproductive processes. In the experiments of Tou et al. [29], flaxseed given at a higher dose (10%) caused earlier puberty onset, higher relative ovarian weight and a lengthened oestrous cycles in rats. Moreover, their offspring, which received secoisolariciresinol diglycoside with their mothers' milk, had increased uterine and ovarian relative weights, earlier puberty, a lengthened oestrous cycle and persistent oestrus. In similar experiments by Jelodar et al. [30] and Mehraban et al. [31], treatment of rats with flaxseed or a flaxseed and spearmint mixture resulted in an increase in the number of primary, pre-antral and antral ovarian follicles, a decrease in the number of cystic follicles, an increase in the thickness of the granulosa cell layer and a decrease in the thickness of the theca layer in rats with polycystic ovarian syndrome. On the contrary, in experiments by Tou et al. [29], the exposure of rats to a diet containing 5% flaxseed or its lignan precursor, secoisolariciresinol diglycoside, reduced ovarian weight, delayed puberty and prolonged the dioestrous phase of the oestrous cycle, demonstrating the inhibitory influence of flaxseed given at this dose and its molecule on rat reproduction. In experiments by Pourjafari et al. [32], feeding with flaxseed decreased the number of rat ovarian follicles.

In the experiments of Pourjafari et al. [33], the feeding of mice with flaxseed reduced the number of healthy ovarian follicles and increased the number of atretic follicles; i.e., in mice flaxseed promoted the atresia of ovarian follicles. In other experiments of this group [34], the dietary extract of flax or flaxseed was able to increase ovarian weight, but it decreased the size of ovarian follicles. On the other hand, in experiments by Vlckova et al. [35, 36], the feeding of mice with flaxseed was able to increase the size of both the ovary and of the ovarian follicles and to promote oestrus.

The dietary flaxseed did not affect growth of goat ovarian follicles isolated and cultured in vitro [37].

The feeding of gilts with flaxseed promoted ovarian growth but suppressed ovarian folliculogenesis [12]

In cows, the dietary flaxseed oil increased the number of ovarian follicles [15]. Ulfina et al. [38] also reported the stimulatory action of flaxseed on bovine ovarian folliculogenesis: it increased the size of the dominant ovarian follicle and corpus luteum, promoted uterine involution during the *post-partum* period and shortened the time up to entrance to the next reproductive cycle. The shortening of *post-partum* period was reported also by Jahani-Mogha-

dam et al. [10]. They observed the ability of flaxseed to decrease the incidence of cystic follicles but observed no changes in the general pregnancy rate. Furthermore, Ambrose et al. [39] reported the ability of a diet enriched in flaxseed alpha-linolenic acid to reduce the incidence of pregnancy losses in cows. All these publications demonstrated the stimulatory action of flaxseed on bovine female reproductive processes. On the other hand, some authors [13, 40] did not observe flaxseed on bovine ovarian folliculogenesis. In the experiments of Zachut et al. [41], the feeding of cows with flaxseed oil even prolonged their oestrous cycle.

Numerous animal and *in vitro* studies [20, 24, 27, 42–45] demonstrated that flaxseed consumption suppresses ovarian cancer development.

There are only two reports concerning flaxseed influence on female reproductive organs besides the ovary. Sacco et al. [46] observed the histomorphological changes in uteri of rats fed with flaxseed, similar to the changes induced by oestrogen, indicating uterine activation and growth. In the experiments of Vlckova et al. [36], the dietary flaxseed increased the thickness of the endometrium and myometrium in mice uteri.

Therefore, the performed animal and *in vitro* studies demonstrated both the stimulatory and inhibitory actions of flaxseed and its molecule on ovarian folliculogenesis and cyclicity. The character of this effect depended on the model object/species and flaxseed dose tested. The influence of flaxseed on other female reproductive organs is possible, but it has not been adequately verified.

Effect on oocytes and embryos

The ability of flaxseed to affect ovarian folliculogenesis, which defines oocyte maturation, and further development indicates a possible flaxseed influence on oo- and embryogenesis. Indeed, dietary flaxseed was able to increase the size of oocytes and their germinal vesicles within primordial ovarian follicles (but not in follicles at other stages of development) in mice [35, 36] and gilts [12]. The feeding of cows with flaxseed oil increased the number of recovered oocytes and their ability to cleave and to reach the blastocyst stage after *in vitro* fertilization [14, 15]. In experiments by Dutra et al. [47], a diet containing flaxseed increased the number and morphological quality of goat embryos. On the other hand, in the experiments of Cadenas et al. [37], the feeding of goats with flaxseed promoted growth of their oocytes but not their fertilization and subsequent cleavage rate.

These observations suggest that flaxseed may have beneficious influence on animal oo- and embryogenesis.

Effect on reproductive hormones

Some animal *in vivo* and *in vitro* studies demonstrated the ability of flaxseed to affect production, metabolism and the reception of steroid hormones in ovarian cells.

Treatment of rats with flaxseed increased the plasma level of progesterone and decreased the level of testosterone and sometimes oestradiol but not of dehydroepiandrosterone [11, 30, 31]. In the experiments of Tou et al. [29], the consumption of flaxseed increased oestradiol levels in rat plasma.

In the experiments of Pourjafari on mice, dietary flaxseed resulted in a decrease in oestradiol in their blood plasma [33]. On the other hand, Vlckova et al. reported the ability of dietary flaxseed to increase concentrations of progesterone and oestradiol in mice plasma [35, 36] and the secretion of these hormones by isolated ovarian fragments [35]. Furthermore, in the experiments of these authors, feeding with flaxseed increased the expression of receptors to FSH and oxytocin in murine ovaries [35] and steroid hormones receptors on murine uteri [36].

In chickens, a flaxseed diet affected oestradiol metabolism by increasing CYP1A1 expression with a corresponding increase in the onco-protective oestradiol metabolite, 2-methoxyestradiol [27, 43, 44]. Furthermore, it decreased the expression of ovarian alpha oestrogen receptors [27, 43].

As concerns cows, Ulfina et al. [38] and Jahani-Moghadam et al. [10] reported increases, whilst Hutchinson et al. [40] reduced progesterone levels in the plasma of cows fed with flaxseed. Hutchinson et al. [40] did not observe flaxseed influence on bovine plasma oestradiol levels. Nevertheless, Zachut et al. [41] reported that the dietary flaxseed tended to reduce oestradiol levels in ovarian follicles. The later studies of this author, however, showed an increase in follicular oestradiol level in cows fed with flaxseed [9].

A flaxseed diet did not affect steroid hormones' levels in porcine plasma [12].

In women, the eating of flaxseed increased serum 2-hydroxyestrone levels [48]. Treatment of patients suffering from polycystic ovarian syndrome decreased total and free testosterone levels in their plasma [49, 50]. On the other hand, Mirmasoumi et al. [51] did not find any changes in plasma steroid hormone levels in patients treated with flaxseed against polycystic ovarian syndrome.

Besides steroid hormones, flaxseed appears to affect peptide hormones. A flaxseed diet decreased LH levels in rat [33] and bovine [11] plasma, and reduced prolactin concentration in women's blood [48]. Dietary flaxseed was able to reduce IGF-I levels in pig plasma [12] and insulin levels in rat blood [11] and to increase IGF-I levels in murine plasma [36] and insulin concentration in women's [49] plasma. On the contrary, subsequent experiments [50–52] showed a decrease in plasma insulin levels in women after flaxseed consumption. In rats, flaxseed suppressed the production of anti-Mullerian hormone by ovarian cells [32]. Flaxseed reduced leptin levels in women's plasma [52] and leptin release by cultured porcine ovarian granulosa cells [53].

Moreover, flaxseed diet reduced the expression of prostaglandin E2 and COX-2, the enzyme responsible for its synthesis in chicken plasma [43]. On the other hand, in cows, dietary flaxseed did not affect the endometrial expression of genes involved in prostaglandin synthesis [40].

Therefore, an amount of evidence demonstrates the ability of flaxseed to act on production, release and reception of hormones and growth factors produced by the pituitary, ovary and uterus, which are involved in endocrine and paracrine/autocrine regulation of female reproductive processes in various species.

Mechanisms of Action on Female Reproductive Processes

Flaxseed constituents responsible for its effects on female reproductive processes

The similarity in inhibitory effects of whole flaxseed and of its lignan precursor, secoisolariciresinol diglycoside, on rat ovarian growth, puberty and oestrous cycle [29] indicates that flaxseed influence on these processes can be due to the presence of secoisolariciresinol diglycoside. Similarly, pregnancy in cows was affected not only by whole flaxseed, but also by its alpha-linolenic acid [39].

Animal and *in vitro* studies [24, 27, 42, 44] demonstrated that flaxseed can suppress ovarian cancer because of the presence of alpha-linoleic acid metabolite docosahexaenoic acid (DHA) and oestrogen metabolite 2-methoxyestradiol. The anti-ovarian cancer and anti-metastatic effect has also another flaxseed metabolite – lignin enterolactone [20].

These observations suggest that flaxseed influence on healthy and cancer ovarian cell functions is determined by the presence of its lignans, alpha-linolenic acid and their products.

Mediators of flaxseed effects on female reproductive processes

The numerous mechanisms and mediators of flaxseed on various physiological processes have been outlined above. Principally, all of them could be involved in mediating the influence of flaxseed and its molecules on female reproductive processes too. Nevertheless, only a few of them were indicated in relation to flaxseed action on female reproduction, whilst the substantial part of the available knowledge concerned flaxseed action not on healthy but on ovarian cancer cells.

Oestrogens are important regulators of female reproduction (ovarian follicullogenesis, oogenesis, embryo implantation in uterus and pregnancy [54]). Previously we mentioned the ability of flaxseed to affect oestradiol release [27, 29, 32, 35, 36, 41, 43, 44], oestradiol metabolism [27, 43, 48], expression of oestrogen receptors [27, 36, 43] and similarity of its action to the action of oestrogen [29]. These data indicate that flaxseed molecules with phytoestrogen activity can affect oestrogen-dependent reproductive processes and ovarian carcinogenesis via oestrogen receptors.

The ability of flaxseed to affect the synthesis of prostaglandin E2 mentioned above [42, 43] indicates the involvement of this prostaglandin in mediating flaxseed effects too.

Furthermore, flaxseed altered the expression of markers of IGF-I- [12, 36] and insulin- [11, 49] signalling pathways, which are known regulators of healthy ovarian cells [54] and their malignant transformation [27].

The involvement of steroid hormones, gonadotropins, leptin, insulin, IGF-I, anti-Mullerian hormone, oxytocin and prostaglandins in the control of female reproductive processes is well-documented [54]. The ability of flaxseed to affect these molecules suggests that these hormones might be extracellular mediators of flaxseed action on female reproduction. Moreover, flaxseed can affect reproductive functions, influencing not only production but also reception of the hormonal regulators of reproduction. At least, the ability of flaxseed to promote the expression of ovarian FSH and oxytocin receptors has been documented [35].

In addition, flaxseed oils are rich in calories, affecting the general metabolic state, which in turn via metabolic hormones affects reproductive processes [55, 56] and their dysfunctions [57]. These facts indicate that flaxseed can affect reproduction via changes in the general metabolic state.

The involvement of several intracellular signalling mechanisms in mediating flaxseed action on female reproduction might be proposed too. The key mediators of flaxseed action could be regulators of ovarian cell proliferation and apoptosis. The addition of flaxseed extract decreased proliferation (accumulation of proliferation marker PCNA) and increased cytoplasmic apoptosis markers (accumulation of Bax) in cultured human ovarian granulosa cells [49]. A similar effect on these markers in ovarian cells was observed in pigs fed with flaxseed diet *in vivo* [12]. Feeding with flaxseed promoted the expression of both proliferation (PCNA, cyclin B1) and apoptosis (bax, caspase 3) markers in murine ovaries [35, 36]. On the other hand, *in vivo* experiments on mice performed by Pourjafari et al. [33] did not show substantial influence of dietary flaxseed on ovarian cell apoptosis and the expression of antiapoptotic molecule bcl-2. *In vivo* experiments of Dixit et al. [27, 43] revealed the ability of a flaxseed diet to induce the apoptosis of chicken ovarian cancer cells. The reported action of flaxseed on intracellular regulators of proliferation and apoptosis in healthy [12, 53] and cancer [24, 27, 43, 45] ovarian cells suggests that its influence on ovarian state and cell turnover could be mediated by its influence on ovarian cell proliferation: the apoptosis rate.

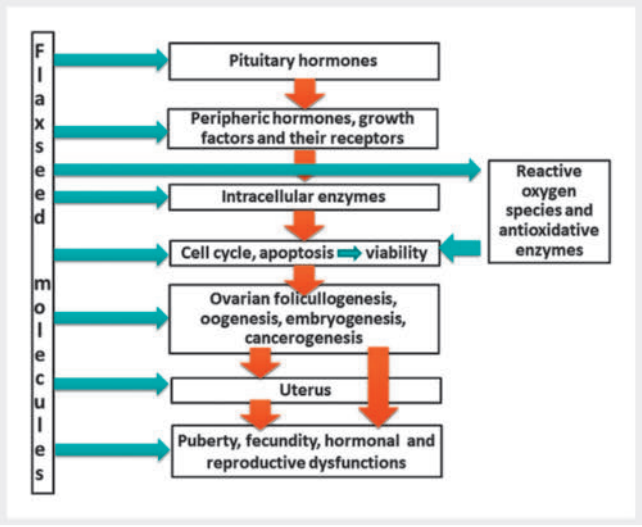
Furthermore, flaxseed decreased the expression of markers of the IGF/insulin pathway (IRS1, IGFBP4 and IGFBP5) and AKT and altered the expression of pro-inflammatory transcription factor NF-kB. All these intracellular pathways could be involved in mediating flaxseed action on ovarian cell proliferation, apoptosis, angiogenesis and tumourgenesis [27].

The flaxseed-derived molecules, 2-methoxyestradiol and DHA can suppress tumour development via promotion of malignant cell apoptosis and suppression of tumour angiogenesis via down-regulation of the p38-MAPK intracellular signalling pathway [24, 45]. The pro-apoptotic action of 2-methoxyestradiol can be partially mediated also by protein kinase C [45].

These reports demonstrate multiple extra- and intracellular pathways mediating the action of flaxseed and its constituents on healthy ovarian functions and their dysfunctions.

Other mediators of flaxseed action on female reproductive processes and their dysfunctions could be proposed. For example, several flaxseed molecules have antioxidant properties, which can be responsible for some flaxseed physiological and curative effects on non-reproductive cells. It is highly probable that flaxseed antioxidants could influence ovarian functions and that they could explain the preventive and therapeutic action of flaxseed on ovarian cancer and other reproductive disorders. Nevertheless, the involvement of antioxidants in mediating flaxseed action on female reproductive processes seems to not have been examined yet.

The available information concerning targets and mechanisms of action of flaxseed and its molecules on female reproductive processes are summarised in ▶ **Fig. 1**.



▶ **Fig. 1** The targets and mechanisms of the actions of flaxseed and its molecules on female reproductive processes. Details are in the text.

Application in reproductive biology and medicine

The results of some basic studies performed on laboratory and farm animals and on cell cultures suggest the applicability of flaxseed for improvement of farm animal reproductive efficiency. As described above, feeding cows and goats with flaxseed can promote the development of their ovarian follicles [15, 38] and increase the number and quality of their oocytes and embryos [14, 15, 24, 47].

The numerous demonstrations of flaxseed effect on steroid and peptide hormones (see above) define puberty [58], ovarian follicular reserve, premature ovarian failure [59] and symptoms of menopause [60, 61]. Furthermore, the flaxseed is able to affect rat puberty [29, 62]. These facts indicate that flaxseed could be potentially applicable for the control of puberty, reproductive age and menopause and for the prevention and treatment of their disorders. Nevertheless, this hypothesis requires pre-clinical and clinical validation.

Furthermore, animal and *in vitro* studies indicated applicability of flaxseed and its constituents for the prevention and treatment of polycystic ovarian syndrome [11, 30, 31, 49] and of ovarian cancer [20, 24, 27, 42–44], although these indices have not been confirmed by the corresponding clinical studies.

Therefore, the available pre-clinical and animal studies indicate the potential practical applicability of flaxseed for the improvement of farm animal reproduction, ovarian cancer, polycystic ovarian syndrome and maybe of other reproductive dysfunctions.

Conclusions and Possible Direction of Future Studies

Flaxseed contains a number of biologically active molecules, which, acting through multiple signalling pathways, can determine the numerous physiological, protective and therapeutic effects of flaxseed. The available publications demonstrate the action of flaxseed and its constituents on female reproductive system – ovarian growth, follicle development, the resulting puberty and reproductive cycles, ovarian cell proliferation and apoptosis, oo- and embryogenesis, hormonal regulators of reproductive processes and their dysfunctions. These effects can be determined by flaxseed lignans, alpha-linolenic acid and their products. Their action can be mediated by changes in general metabolism, metabolic and reproductive hormones, their binding proteins, receptors and several intracellular signalling pathways including protein kinases, transcription factors regulating cell proliferation, apoptosis, angiogenesis and malignant transformation. Flaxseed and its active molecules are found potentially useful for the improvement of farm animal reproductive efficiency and in the treatment of polycystic ovarian syndrome and ovarian cancer.

On the other hand, a number of important details of flaxseed action on female reproduction and other processes require further elucidation. The flaxseed constituents and mediators of their action responsible for some effects remain to be determined. The published data concerning the character of flaxseed action on many female reproductive processes in various species and even in one model are contradictory. The main data concerning flaxseed effects are obtained in laboratory animal and *in vitro* studies, sometimes on models with dysfunctions (cancer or polycystic ovarian syndrome), whilst the reports of clinical studies are rare and sometimes inconsistent and inconclusive. The contradictory evidence concerning flaxseed effects on female reproductive processes could be explained by different doses and qualities of the tested preparations and the species-specific differences in flaxseed digestion and metabolism, as well as the variation in initial reproductive and metabolic state of the experimental models.

Therefore, the available information suggests the substantial physiological and therapeutic potential of flaxseed but also the need for more profound basic and applied studies of its physiological, protective and therapeutic effects.

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Conflict of Interest

The authors declare that they have no conflict of interest. He wishes to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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