Rooibos (*Aspalathus linearis*) and its Constituent Quercetin Can Suppress Ovarian Cell Functions and Their Response to FSH

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Summary

Rooibos (*Aspalathus linearis* Brum. f) can directly influence female reproduction, but whether rooibos can influence the response of ovarian cells to FSH and whether the rooibos effects are due to the presence of quercetin remain unknown. We compared the influence of rooibos extract and quercetin (both at 10 μg/ml^{-1}) on porcine ovarian granulosa cells cultured with and without FSH (0, 1, 10 or 100 ng/ml^{-1}). The expression of intracellular proliferation (PCNA, cyclin B1) and apoptosis (bax, caspase 3) markers in the cells was detected by immunocytochemistry. The release of progesterone (P), testosterone (T) and estradiol (E) were evaluated with ELISAs. Administration of both rooibos and quercetin reduced the accumulation of proliferation markers and promoted the accumulation of apoptosis markers and the release of T and E. Rooibos stimulated, but quercetin inhibited, P output. Administration of FSH increased the accumulation of proliferation markers, decreased the accumulation of apoptosis markers, promoted the release of P and T, and had a biphasic effect on E output. The addition of both rooibos and quercetin mitigated or prevented the main effects of FSH. The present observations suggest a direct influence of both rooibos and quercetin on basic ovarian functions – proliferation, apoptosis, steroidogenesis and response to FSH. The similarity in the major effects of rooibos and its constituent quercetin indicates that quercetin could be the molecule responsible for the main rooibos effects on the ovary. The potential anti-reproductive effects of rooibos and rooibos constituent quercetin, should be taken into account in animal and human nutrition.

Key words

Ovary • Proliferation • Apoptosis • FSH • Steroid hormone

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Introduction

Rooibos (*Aspalathus linearis* Brum. f) tea is a known health-promoting drink, and its popularity is currently growing [1]. The physiological and therapeutic effects of rooibos have been documented. It can prevent the signs of metabolic syndrome (oxidative stress, type 2 diabetes mellitus, cardiovascular diseases and obesity) and Alzheimer's disease; activate immunocompetent cells; harm some liver and kidney indices and epididymal morphology; but improves sperm concentration, viability and motility; and improves memory [1-3].

There is evidence of direct action of rooibos on basic ovarian cell functions. The addition of rooibos extract at doses of 1, 10 and 100 μg/ml medium was able to inhibit proliferation (downregulated PCNA, cyclin B1 and their mRNAs), promote apoptosis (accumulation of bax) and suppress the release of progesterone (P) and leptin in cultured porcine ovarian granulosa cells [4]. However, subsequent studies did not confirm the ability of rooibos additions at a dose of 10 μg/ml to affect this apoptosis marker [3,4]. Furthermore, in these studies, rooibos additions was able to promote the accumulation of PCNA [5,6] and either to promote [7] or reduce [6] P release. Rooibos was able to prevent the toxic effects of benzene [7] and xylene [5] on porcine ovarian granulosa cells.

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Overall, the results of *in vitro* studies performed in cultured porcine ovarian granulosa cells suggest an ability of rooibos to directly affect ovarian cell proliferation, apoptosis and hormone release. On the other hand, the available evidence concerning the effect of rooibos on ovarian cells is contradictory and requires further verification. Whether rooibos can modify the ovarian cell response to gonadotropins remains unknown.

The molecule(s) responsible for the reproductive effects of rooibos remain to be identified. One such rooibos biological active molecule might be quercetin (3,3',4',5,7-pentahydroxyflavanone or 3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one). Quercetin and its metabolites are the major flavonoids contained in rooibos and other plants consumed by humans and animals [8,9]. Quercetin is presented in rooibos tea in amount more than 5 mg/kg [10-12]. This molecule has antioxidant and anti-inflammatory properties, the ability to regulate cell proliferation, and cytotoxicity against cancer cells [9,13,14].

Both pro- and anti-reproductive properties of quercetin have been reported. *In vivo* experiments demonstrated an ability of quercetin to stimulate female reproductive processes. Dietary quercetin increased mouse ovarian weight; promoted mouse ovarian follicullogenesis [15]; increased the proportion of ovarian primordial follicles [16,17] and secondary follicles; decreased the number of mature follicles, atretic follicles and corpora lutea; and disrupted the mouse estrous cycle [16]. Quercetin reduced the number of atretic and cystic follicles and increased the number of healthy follicles in rats [18-20], rabbits [21] and humans [22,23]. Quercetin also supported the health and development of cultured bovine ovarian follicles [24]. Dietary quercetin reduced the number of apoptotic ovarian cells in rabbit [21], mouse [17] and rat [19,20,25] ovaries. Finally, experiments on rats and mice demonstrated an ability of quercetin to prevent signs of premature ovarian failure [25], polycystic ovarian syndrome [26], ovarian aging [14] and the reproductive toxicity of xylene [27], benzene [28] and metal nanoparticles [7]. Furthermore, quercetin addition reduced the expression of apoptotic markers in rat ovaries [25, 26], as well as in cultured bovine [27,29] and porcine [27-30] granulosa cells. Another study [31] reported the stimulatory action of quercetin on P release by cultured porcine granulosa cells.

On the other hand, some studies have demonstrated direct anti-proliferative and pro-apoptotic effects of quercetin on ovarian cells. Dietary quercetin reduced the accumulation of proliferation markers or their transcripts in mouse ovaries [16] and in cultured bovine [27,29] and porcine [27-31] granulosa cells. Several authors observed suppressive action of quercetin on steroid hormone synthesis and release by rat ovaries [25,26] and cultured bovine [30] and porcine [7,8,29,30] ovarian cells.

Quercetin was able to downregulate not only basic ovarian functions but also the response of ovarian cells to the upstream hormonal regulator the gonadotropin FSH. The ability of FSH to promote ovarian cell proliferation and follicullogenesis, oogenesis and steroidogenesis and to suppress ovarian cell apoptosis is well documented [32]. The addition of quercetin was able to prevent the action of FSH on proliferation and apoptosis in cultured porcine ovarian granulosa cells [30].

Thus, the available data concerning the characteristics of quercetin and its effects on basic ovarian cell functions (proliferation, apoptosis, steroidogenesis) remain contradictory and require further verification. Whether the constituent quercetin is responsible for the reproductive effects of rooibos remains unknown.

The aim of the present study was to examine whether rooibos can affect basic ovarian cell functions and their response to the hormonal stimulator FSH and whether the rooibos effects are due to the presence of quercetin. To address these questions, we compared the influence of rooibos extract and quercetin on porcine ovarian granulosa cells cultured with and without FSH given at different doses.

Materials and Methods

Isolation and culture of granulosa cells

Granulosa cells were isolated from the ovaries of noncycling pubertal gilts (approximately 180 days of age) and cultured as described previously [4-7,27-30,33]. The aspirated granulosa cells were precultured at a final concentration of 10^6 cells/ml in sterile Dulbecco's modified Eagle's medium/F12 1:1 medium supplemented with 10 % fetal calf serum (both from BioWhittakerTM, Verviers, Belgium) and 1 % antibiotic-antimycotic solution (Sigma, St. Louis, MO, USA) in 16-well chamber slides and 24-well plates (Nunc Inc., International, Naperville, USA, 200 µl or 2 ml per well, respectively) for 3 days. Thereafter, the medium was replaced with a medium of the same composition with or without porcine FSH (Sigma, 0, 1 10 or 100 ng/ml

medium) alone or in the presence of rooibos (*Aspalathus linearis* Brum. f., organic 100 % pure red tea of single origin from rooibos superior leafs, short cuts, GMO and caffeine free, Clanwilliam, South Africa, 10 μg/ml) or quercetin (AppliChem GmbH, Darmstadt, Germany, 10 μg/ml). These doses correspond to the effective doses of FSH [29,33], rooibos [5,6] and quercetin [7,8,27-31] used in previous *in vitro* experiments. FSH was dissolved in the incubation medium immediately before experiments. The dry rooibos and quercetin powders were first dissolved in 50 μl dimethyl sulfoxide (DMSO) to obtain a stock solution of 1 mg/ml plant extract. After these stock solutions were dissolved in culture medium immediately before their addition to the cells, the final concentration of DMSO did not exceed 0.001 %. Our previous studies demonstrated that DMSO at this dose does not affect cell viability or other parameters (not shown). Controls included ovarian cells cultured in the incubation medium (with 0.001 % DMSO) without the presence of any treatment and medium incubated without cells (blank control). After two days of culture, the cells and incubation medium were analyzed as described below. Cell viability was determined before and after culture using Trypan blue staining. In each case, cell viability ranged from 70 % to 80 %, and no significant differences were observed in these indices between the control and experimental groups

Immunocytochemical analysis

Following washing and fixation, the cells were incubated in blocking solution (1 % goat serum in phosphate-buffered saline (PBS)) at +20 °C for 1 h to block nonspecific binding of antiserum. Next, the cells were incubated in the presence of mouse monoclonal antibodies against PCNA, cyclin B1 (promoters and markers of cell cycle, proliferation and DNA repair [34,35], bax, and caspase 3 (promoters and markers of cytoplasmic apoptosis; [36]) (all from Santa Cruz Biotechnology, Inc., Dallas, TX, USA, dilution 1:500 in PBS) for 2 h at +20 °C. To detect the binding sites of the primary antibody, the cells were incubated in secondary swine or goat antibody against mouse IgG labeled with fluorescein isothiocyanate (FITC) or horseradish peroxidase (both from Sevac, Prague, Czech Republic, dilution 1:1000) for 1 h. FITC was detected *via* fluorescence microscopy. DAB was visualized by staining with DAB substrate (Roche Diagnostics GmbH, Manheim, Germany). Following DAB staining, the cells on chamber slides were washed in PBS and covered with a drop of Glycergel Mounting Medium (DAKO, Glostrup, Denmark), and the coverslip was attached to a microslide. The presence and localization of PCNA and bax positivity in cells were demonstrated based on FITC green fluorescence or DAB-peroxidase brown staining. Cells processed without the primary antibody were used as a negative control. The ratio of DAB-HRP-stained cells to the total cell number was calculated. The presence of PCNA, cyclin B1, bax and caspase 3 in the cells after culture is documented in Figure 1.

Hormone immunoassay

The concentrations of P, testosterone (T), and 17β-estradiol (E) were determined in 25 μl aliquots of incubation medium. The hormones were assayed using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (LDN Immunoassays and Services, Nodhorn, Germany). Assays of hormone concentration in the incubation medium were performed in duplicate. The rates of substance secretion were calculated per 10^6 viable cells/day.

Antiserum against progesterone cross-reacted ≤1.1 % with 11-desoxycorticosterone, ≤0.35 % with pregnenolone, ≤0.30 % 17α-OH with progesterone, ≤0.20 % with corticosterone, <0.10 % with estriol, 17β-estradiol, testosterone, cortison and 11-desoxycortisol, <0.02 % with DHEA-S and cortisol. Sensitivity of the assay was 0.045 $ng.ml^{-1}$. Intra- and inter-assay coefficients of variation did not exceed 5.4 % and 5.59 %, respectively (Table 1).

The cross-reactivity of antiserum against testosterone was ≤3.3 % with 11β-hydroxyestosterone and 19-nortestosterone, ≤0.9 % with androstenedione, ≤0.8 % with 5α-dihydrotestosterone, <0.1 % with 17α-methyltestosterne, epitestosterone, oestradiol, progesterone, cortisol, oestrone and danazol. The maximal intra- and inter-assay coefficients of variation were 4.16 % and 4.73 %, respectively. Sensitivity of the assay was 0.083 $ng.ml^{-1}$.

The sensitivity of the 17β-estradiol assay was 6.2 $pg.ml^{-1}$. Intra- and inter-assay coefficients of variation did not exceed 6.4 % and 4.5 %, respectively. The cross-reactivity of antiserum against 17β-estradiol was less than ≤9.5 % with fulvestrant, ≤4.2 % with estrone, ≤3.8 % with E2-3-glucuronide, ≤3.6 % with E2-3-sulphate, ≤0.4 % with estriol, <0.1 % with androstenedione, 17-hydroxyprogesterone, corticosterone, pregnenolone, E2-17-glucuronide, progesterone and testosterone.

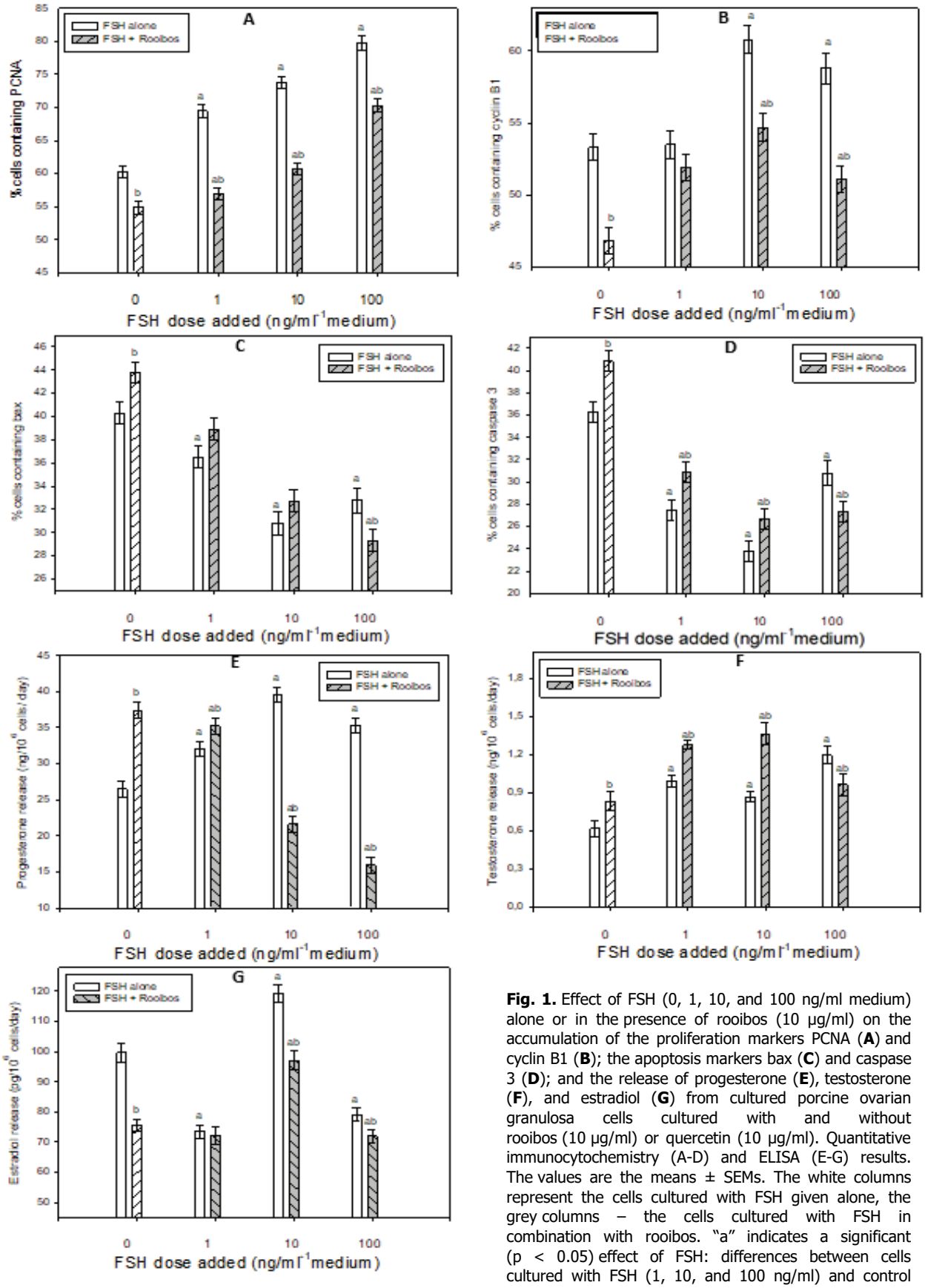

Fig. 1. Effect of FSH (0, 1, 10, and 100 ng/ml medium) alone or in the presence of rooibos (10 μg/ml) on the accumulation of the proliferation markers PCNA (**A**) and cyclin B1 (**B**); the apoptosis markers bax (**C**) and caspase 3 (**D**); and the release of progesterone (**E**), testosterone (**F**), and estradiol (**G**) from cultured porcine ovarian granulosa cells cultured with and without rooibos (10 μg/ml) or quercetin (10 μg/ml). Quantitative immunocytochemistry (A-D) and ELISA (E-G) results. The values are the means ± SEMs. The white columns represent the cells cultured with FSH given alone, the grey columns – the cells cultured with FSH in combination with rooibos. "a" indicates a significant ($p < 0.05$) effect of FSH: differences between cells cultured with FSH (1, 10, and 100 ng/ml) and control cells (FSH at 0 ng/ml). "b" indicates a significant ($p<0.05$) effect of additive of rooibos: differences between the corresponding groups of cells cultured with and without rooibos.

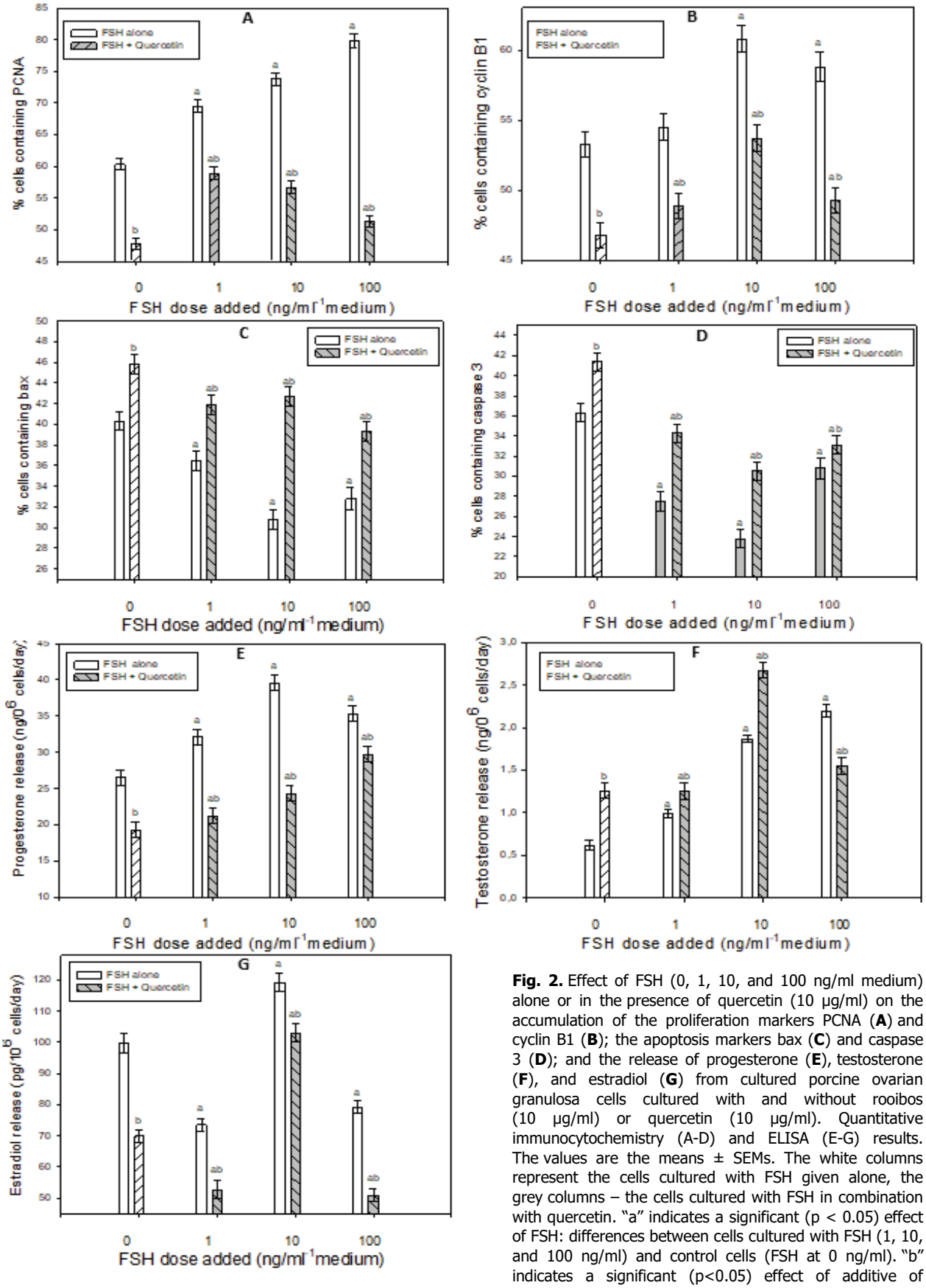

Fig. 2. Effect of FSH (0, 1, 10, and 100 ng/ml medium) alone or in the presence of quercetin (10 μg/ml) on the accumulation of the proliferation markers PCNA (**A**) and cyclin B1 (**B**); the apoptosis markers bax (**C**) and caspase 3 (**D**); and the release of progesterone (**E**), testosterone (**F**), and estradiol (**G**) from cultured porcine ovarian granulosa cells cultured with and without rooibos (10 μg/ml) or quercetin (10 μg/ml). Quantitative immunocytochemistry (A-D) and ELISA (E-G) results. The values are the means ± SEMs. The white columns represent the cells cultured with FSH given alone, the grey columns – the cells cultured with FSH in combination with quercetin. "a" indicates a significant ($p < 0.05$) effect of FSH: differences between cells cultured with FSH (1, 10, and 100 ng/ml) and control cells (FSH at 0 ng/ml). "b" indicates a significant ($p<0.05$) effect of additive of quercetin: differences between the corresponding groups of cells cultured with and without quercetin.

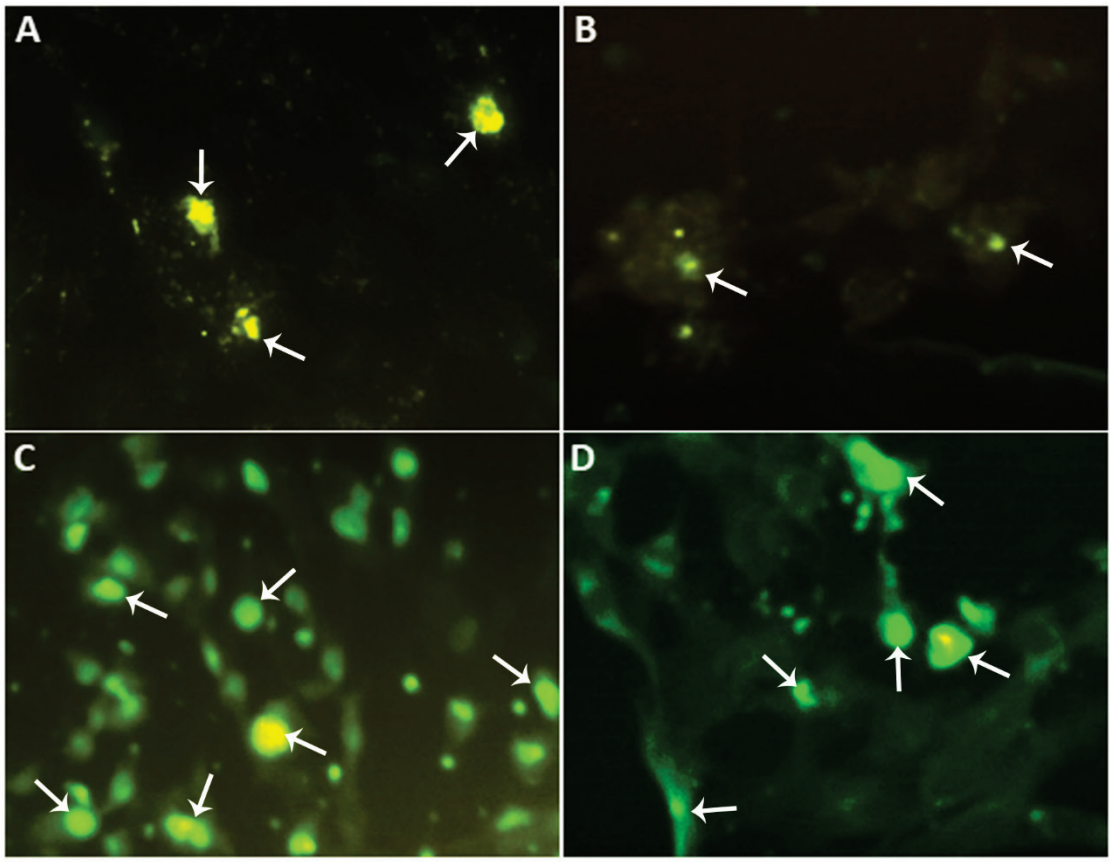

Fig. 3. Immunocytochemistry images of cultured porcine granulosa cells containing PCNA (**A**), cyclin B1 (**B**), bax (**C**) and caspase 3 (**D**) detected by labeling with FITC (green fluorescence, indicated by arrows). Scale bars: 1 cm =10 μm.

Table 1. Characteristics of immunoassays of hormones

Measured hormone	Specificity (cross-reactivity)	Sensitivity (ng/ml)	Coefficient of variation (%)	
			Intra-assay	Inter-assay
Progesterone	≤1.1 % to 11-desoxycorticosterone, ≤0.35 % to pregnenolone, ≤0.30 % 17α-OH to progesterone, ≤0.20 % to corticosterone, <0.10 % to estriol, 17β-estradiol, testosterone, cortisone and 11-desoxycortisol, <0.02 % to DHEA-S and cortisol.	0.045	<5.4	<5.59
Testrosterone	≤3.3 % to 11β-hydroxyestosterone and 19-nortestosterone, ≤0.9 % to androstenedione, ≤0.8 % to 5α-dihydrotestosterone, <0.1 % to 17α-methyltestosterne, epitestosterone, oestradiol, progesterone, cortisol, oestrone and danazol	0.083	< 4.16	< 4.73
Estradiol	≤9.5 % to fulvestrant, ≤4.2 % to estrone, ≤3.8 % to E2-3-glucuronide, ≤3.6 % to E2-3-sulphate, ≤0.4 % to estriol, <0.1 % to androstenedione, 17-hydroxyprogesterone, corticosterone, pregnenolone, E2-17-glucuronide, progesterone and testosterone.	6.2	< 6.4	< 4.5

Statistical analysis

The data shown are the means of values obtained in at least three separate experiments performed on separate days with separate groups of granulosa cells, each obtained from at least 10 animals. Each experimental group of granulosa cells was represented by three chamber slide wells and by four culture plate wells. The proportions of granulosa cells containing specific immunoreactivity were calculated from at least 1000 cells per chamber. Hormonal assays in incubation medium were performed in duplicate. The blank control values were subtracted from the value determined by EIA/RIA in cell-conditioned medium to exclude any nonspecific background (less than 14 % of total values). The rates of hormone secretion were calculated using 1×10^6 viable cells/day. Significant differences between the experiments and groups were evaluated with one- or two-way ANOVA followed by Duncan's test using SigmaPlot 11.0 software (Systat Software, GmbH, Erkrath, Germany). The present values represent means + SEM. In the results of this study, the following comparisons between selected groups are presented: a) the difference between cells cultured without or with FSH and b) the difference between corresponding groups of cells cultured without or with rooibos or quercetin. $p<0.05$ was considered statistically significant.

Results

Effect of FSH

Comparison of cells cultured with and without FSH demonstrated the influence of this hormone on all measured cell parameters. FSH increased the percentage of cells containing PCNA (at all doses added, Fig. 1A, 2A) and cyclin B1 (at doses of 10 and 100 ng/ml, Fig. 1B, 2B). Furthermore, FSH given at all doses reduced the proportion of cells containing bax (Fig. 1C, 2C) and caspase 3 (Fig. 1D, 2D). Administration of FSH at all doses stimulated the release of P (Fig. 1E, 2E) and T (Fig. 1F, 2F). FSH at doses of 1 and 100 ng/ml reduced the release of E, but FSH addition at a dose of 10 ng/ml resulted in increased E output (Fig. 1G, 2G).

Effect of rooibos and quercetin

Analysis of cells cultured without FSH (FSH dose 0 ng/ml) demonstrated the influence of both rooibos and quercetin (both at 10 µg/ml) on basic ovarian cell indices (Fig. 1, 2 and 3). Both additives significantly reduced the percentage of cells containing PCNA (Fig. 1A, 2A, 3A) and cyclin B1 (Fig. 1B, 2B, 3B) and increased the proportion of cells containing bax (Fig. 1C, 2C, 3C) and caspase 3 (Fig. 1D, 2D, 3D). The addition of rooibos promoted and quercetin decreased P release (Fig. 1E, 2E). Both additives increased the release of T (Fig. 1F, 2F), but decreased E output (Fig. 1G, 2G).

Ability of rooibos and quercetin to modify FSH effects

Finally, comparison of the effects of FSH alone and FSH in combination with plant additives demonstrated the ability of both rooibos and quercetin to modify FSH action. Both plant additives reduced the stimulatory action of FSH given at all doses on the accumulation of PCNA (Fig. 1A, 2A) and cyclin B1 (Fig. 1B, 2B). Furthermore, both rooibos and quercetin reduced the inhibitory influence of FSH on the accumulation of bax (at doses of 1 and 100 ng/ml and at all doses, respectively, Fig. 1C, 2C). Both rooibos and quercetin mitigated the FSH action on the accumulation of caspase 3 at all doses added (Fig. 1D, 2D). The presence of rooibos promoted the stimulatory action of FSH given at 1 ng/ml on P release but prevented and even inhibited the action of FSH given at 10 or 100 ng/ml. Administration of quercetin reduced but did not completely prevent the stimulatory action of FSH (at all doses) on P output (Fig. 1E, 2E). Rooibos promoted the stimulatory action of FSH given at 1 and 10 ng/ml on T release but reduced this action of FSH given at 100 ng/ml. Similarly, quercetin increased the T-promoting effect of FSH given at 1 and 10 ng/ml but reduced this effect of FSH when added at 100 ng/ml (Fig. 1F, 2F). The presence of rooibos reduced the stimulatory effect of FSH given at 10 ng/ml but promoted FSH inhibitory action at 100 ng/ml on E output, while in the presence of quercetin, the inhibitory effect of FSH (at 1 and 100 ng/ml) was increased, but its stimulatory action (at dose 10 ng/ml) was reduced (Fig. 1G, 2G).

Discussion

FSH as a regulator of ovarian cell functions

In the present study, administration of FSH increased the accumulation of the proliferation markers PCNA and cyclin B1 in cultured cells. PCNA is considered a marker and promoter of the cell cycle S-phase, and cyclin B1 is considered a driver of the cell cycle G-phase [34,35]. Therefore, FSH can promote cell proliferation *via* the upregulation of stimulators of both the S- and G-phases of the cell cycle. Furthermore, in the

present study, FSH reduced the accumulation of bax and caspase 3, two apoptosis promoters and markers [36]. Furthermore, FSH stimulated the release of P and T. These observations are in line with the available data concerning the physiological role of FSH as a promoter of ovarian cell proliferation and a suppressor of ovarian cell apoptosis and the resulting ovarian follicular growth [32]. Furthermore, these observations confirm previous evidence indicating a role of FSH as a regulator of ovarian steroidogenesis [32]. Probably it is the first observation of the biphasic effect of FSH on E release by ovarian cells. Previously such biphasic influence of gonadotropins FSH was observed only on adenylate cyclase [37], insulin-like growth factor binding protein 3 [38], on Kit ligands in mice oocytes [39] and expression of sirtuin 1 in porcine granulosa cells [33], but not on ovarian steroid hormones output.

The biphasic action of FSH on E release suggests a dose-dependent effect of FSH on ovarian cells: particular doses of FSH can promote the development of healthy ovarian follicles (which are characterized and stimulated by high estradiol levels), but FSH at other doses can stimulate follicular atrophy (which is characterized and promoted by high androgen and low estrogen production) or luteinization (which is characterized by high progestogen and low estrogen production) [32].

Rooibos and quercetin as regulators of ovarian cell functions

In the present study, both rooibos and quercetin reduced the accumulation of PCNA and cyclin B1 and increased the accumulation of bax and caspase 3. These observations suggest that these plant additives can suppress ovarian cell proliferation at both the S- and G-phases of mitosis [34,35] and promote apoptosis [36] (see above). Such changes indicate that both rooibos and quercetin can suppress ovarian follicular growth and induce follicular atresia *via* downregulation of cell proliferation and upregulation of apoptosis. On the other hand, both additives promoted the release of T and diminished the release of E. T is a marker and promoter of follicular atresia [32]. Therefore, the possibility that the inhibitory action of the plant additives on ovarian cell proliferation and apoptosis is mediated by upregulation of androgen production cannot be excluded. On the other hand, these additives suppressed the release of E, which is considered a marker and promoter of ovarian follicular health and growth [32]. The possibility that the effects of plant additives depend not (or not only) on the production of one particular hormonal mediator but also on their proportion and functional interrelationships cannot be excluded. The possible explanation for the hormonal mechanisms by which plant additives influence ovarian cells is also complicated by the opposite action of rooibos and quercetin on P release: rooibos stimulated P output, but quercetin inhibited it. Understanding the causes and physiological significance of the influence of plant additives on ovarian hormones requires further investigation. These additives may not only have an impact on hormone release but also on hormone synthesis, conversion of progestogen to androgen and androgen to estrogen, hormone reception and metabolism. These processes, which were not analyzed in the present study, could be the subject of future studies.

The present observations confirm previous findings concerning the suppressive direct action of rooibos [4, 6] and quercetin [7,8,16,25-31] on ovarian cells. On the other hand, the recent observations are not in line with reports concerning the ability of quercetin to promote rodent [14-20,25], rabbit [21], bovine [24] and human [22,23] ovarian functions *in vivo*. Furthermore, the results are not in line with previous publications concerning the anti-apoptotic action of quercetin on bovine [27,29] and porcine [27-30] granulosa cells and its stimulatory action on proliferation and steroidogenesis in cultured porcine granulosa cells [5,6,30].

These data demonstrate the ability of both rooibos and quercetin to influence female reproductive functions *via* direct action on ovarian cells. On the other hand, the nature of their influence might depend on the species and on individual variations in reproductive and physiological states, which are not always easy to identify. Nevertheless, the present study provides strong evidence of the suppressive action of rooibos and quercetin on porcine ovarian granulosa cell functions.

The effects of rooibos and quercetin on 6 of the 7 parameters analyzed in the present study were similar. Only the influence of these additives on progesterone release was different. The high amount of quercetin in rooibos and the similarity of the main rooibos and quercetin effects on ovarian cells reported previously (see Introduction) and observed in the present study indicate that quercetin may be the key constituent defining the influence of rooibos on ovarian functions.

Rooibos and quercetin as modulators of FSH action on ovarian cell functions

In the present study, both rooibos and quercetin were found to not only affect basic ovarian cell functions but also to modify the stimulatory action of FSH on these functions. Both plant additives reduced the stimulatory action of FSH on proliferation markers and the inhibitory action of FSH on apoptosis markers. The effect of both FSH and plant additives on steroid hormone release was sometimes dependent on the administered dose, but both rooibos and quercetin mainly reduced or prevented FSH action on steroid hormone release. This confirms our previous report [30] concerning the ability of quercetin to prevent the action of FSH on proliferation and apoptosis in cultured porcine ovarian granulosa cells. This study provides the first evidence of the ability of rooibos to prevent FSH action on ovarian functions.

The present observations suggest an additional mechanism of action of rooibos and quercetin in female reproduction – these substances can not only directly affect basic ovarian cell functions (proliferation, apoptosis, and hormone release) but also modify (mainly prevent) the response of ovarian cells to the main upstream hormonal stimulator FSH.

The mechanism(s) underlying the influence of rooibos and quercetin on FSH action require further clearance. An influence of plant substances on the number or activity of FSH receptors or on the postreceptor mechanisms of FSH action might be suggested. This suggestion is supported by the influence of quercetin on FSH receptors and some postreceptor mediators of FSH action in rat ovaries [25]. Another possible explanation is the phytoestrogenic activity of quercetin. Quercetin can bind and downregulate estrogen receptors [40], which play an important role in the promotion of female reproductive events and the generation of FSH receptors [32].

Conclusions

The present results further demonstrate the influence of rooibos and quercetin on ovarian functions. They suggest a direct, predominantly suppressive, influence of these plant additives on basic ovarian functions – proliferation, apoptosis, and steroidogenesis. In addition, this study demonstrated a novel mechanism by which rooibos and quercetin act on these functions – suppression of the ovarian cell response to the stimulatory action of the physiological hormonal regulator FSH. The similarity in the major effects of rooibos and its constituent quercetin indicates that quercetin could be the molecule responsible for the main effects of rooibos on the ovary.

On the other hand, some aspects of the effects of rooibos and quercetin on female reproduction require further elucidation. The causes of the controversies in the available information concerning the reproductive effects of these substances have not been explained. The main relevant studies were performed in cell cultures. These plant additives have not been tested on pigs or humans under *in vivo* conditions. Nevertheless, the potential anti-reproductive effects of rooibos, food or drinks containing rooibos and rooibos constituents, such as quercetin, should be taken into account in animal and human nutrition.

Conflict of Interest

There is no conflict of interest.

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