Genistein crosses the bioartificial oviduct and alters secretion composition

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16 *in vitro* Derived Oviduct Fluid

17 **Abbreviations**

18 *iv*DOF — *in vitro* derived oviduct fluid

19 BOEC — bovine oviduct epithelial cell

20 G7G — genistein-7-glucoside

1. Introduction

Genistein-7-glucoside [G7G (Figure 1A)] is a natural conjugated isoflavone — *i.e.* a plant derived polyphenolic molecule capable of exerting oestrogenic effects; otherwise known as a phytoestrogen. G7G is found in a range of foods including lentils, soybeans, and coffee [1,2]. Following ingestion, G7G is hydrolysed in the intestine [3] to release the aglycone form, genistein (Figure 1B), which is rapidly absorbed by the upper intestine [4] and circulates readily in the blood [5,6].

Soya derivatives are a major component of several foodstuffs, including human milk-replacers, which are high in genistein-7-glucoside. Furthermore, soy has long been popular in eastern societies, with intake increasing rapidly in western societies [7]. In dietary supplements and extracts, the content of isoflavone aglycones as a percentage of total isoflavones can range from 15% to 85% [8]. Furthermore the rising inclusion of soy in processed foods represents the new primary source of isoflavones in UK diets [7,9].

Unlike G7G, [10], the unconjugated flavonoid genistein has been observed in the blood in concentrations of approximately 20 $ng \cdot ml^{-1}$ [8] which is unsurprising given the 1998 UK Total Diet Survey estimated that the average adult daily intake of genistein is 3 $mg \cdot day^{-1}$.

Flavonoids such as genistein reportedly exert numerous beneficial physiological effects [11-13]. For example, genistein can lower blood pressure by up-regulating nitric oxide (NO) synthesis in the vascular endothelium [14-16]. Furthermore, genistein inhibits cell growth and MAP kinase activity in aortic smooth muscle [17], increases TGF-β secretion [18], and is implicated in antioxidant protection of DNA

and low-density lipoprotein, and systemic processes such as the modulation of inflammation, inhibition of platelet aggregation, and modulation of adhesion receptor expression [19-23].

The diverse biological activity of genistein is generally assumed to be a consequence of its chemical structure (Figure 1B), which resembles 17β-oestradiol (E2) (Figure 1C). Genistein can bind to oestrogen receptors (ERs) *in vivo* and is able to exert modest oestrogenic effects [24]. It can promote dimerisation of ERs and subsequent DNA binding at oestrogen response elements (EREs), similarly to E2, thus modulating gene expression [25]. The oestrogenic potency of genistein on ERα and and ERβ is 198% and 182% of that of E2 respectively [26] Genistein has been shown to be effective at activating oestrogen receptors *in vitro* at 1 mM [16].

Although the reproductive significance of E2 is well established, the effects of genistein on reproductive physiology are less well understood. Newbold and coworkers [27] reported that mice treated neonatally (Days 1-5) with genistein *in vivo* developed uterine adenocarcinoma at 18 months. Since then the same group has shown that circulating maternal genistein perturbs the implantation process [28] in addition to disrupting the ability of the oviduct to support physiological embryo cleavage [29]. In a similar study Jefferson and coworkers [30] found that feeding mice with genistein perturbed the expression of several immune response genes in the oviduct epithelium, a consequence of which was an increased in embryo cleavage and a decreased ratio of trophectoderm to inner cell mass cells in the developing offspring *in vivo*. Although this did not affect full term development after embryo transfer, these cumulative findings highlight the potential of genistein to compromise offspring health. In spite of these studies, the presence of genistein in the female reproductive

68 tract has never been confirmed.

69 Building on these findings, the *in vitro* oviduct model recently established by
70 Simintiras and coworkers [31] has been used to investigate whether effects of dietary
71 genistein on the developing conceptus may be direct or indirect. The research
72 questions of this study therefore are: (***a***) does genistein traverse the oviduct
73 epithelium, thereby permitting a direct effect on the embryo? (***b***) Does genistein
74 supplementation impact the amino acid composition of *in vitro* derived oviduct fluid
75 (*iv*DOF), thus potentially affecting the embryo indirectly? (***c***) If so, is the amino acid
76 profile similar to that of E2?

2. Materials and Methods

Unless stated otherwise all reagents were purchased from Sigma Aldrich UK.

2.1. Tissue Harvest

Abattoir-derived bovine reproductive tracts were transported to the laboratory at room temperature in Hank's Buffered Salt Solution (HBSS) (without $CaCl_2$ and $MgCl_2$) supplemented with 10 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) and 1 μM Aprotonin. Primarily stage II [32] reproductive tracts reached the laboratory within 90 minutes of slaughter. Whole oviducts were dissected and cell isolation was performed manually into petri dishes containing HBSS without $CaCl_2$ and $MgCl_2$. The cell suspension was centrifuged for 5 mins at 400 x *g* at room temperature. The supernatant and upper layer containing erythrocytes was discarded and the pellet resuspended in 10 ml HBSS and further centrifuged for 5 minutes at 350 x *g*. Pellets were resuspended in 1 ml culture medium – consisting of 1:1 Dulbecco's Modified Eagle's Medium DMEM and Nutrient Mixture F12 Ham; supplemented with 265 U/ml Penicillin-Streptomycin, 20 μg/ml Amphotericin B, 2 mM L-Glutamine, 2.5% (*v*/*v*) Newborn Calf Serum (NCS), 2.5% (*v*/*v*) Foetal Bovine Serum (FBS), and 0.75% (*w*/*v*) Bovine Serum Albumin (BSA). Similarly to Simintiras and coworkers [31] bovine oviduct epithelial cells (BOECs) were subsequently isolated from fibroblasts based on their differential adhesion times — cells were initially seeded together in T75 flasks and following 18 hours of culture, BOECs (un-adhered) were removed [33].

2.2. BOEC Culture

Isolated BOECs were directly seeded onto the apical fascia of 24 mm Corning Transwell™ 0.4 μM pore Polyethylene Terephthalate (PET) cell culture inserts coated with 10 μg/ml laminin at a density of 1 x 10^6 cells/ml/insert. BOECs were subsequently maintained between two culture media-filled chambers; apical and basal, with 2 ml culture medium in each compartment, at 39°C in 5% CO2, 95% air. Apical and basal media were replaced every 48 hours and a polarised confluent monolayer was achieved after ~ 7 days as determined by Transepithelial Electrochemical Resistance.

2.3 Transepithelial Electrochemical Resistance

Transepithelial Electrochemical Resistance (TEER) was measured using an Evom voltmeter fitted with handheld chopstick electrodes (World Precision Instruments). The TEER of a tight BOEC monolayer was between 700 Ω/cm^2 to 1000 Ω/cm^2 [31,34,35]. In addition to assessing monolayer confluence prior to experimentation, TEER was utilised as a measure of post-treatment cellular integrity.

2.4. *In vitro* Derived Oviduct Fluid

Upon reaching confluence BOECs were cultured in an apical-basal air-liquid interface. The basal medium comprised 2 ml of culture medium while the apical compartment comprised moist air in 5% CO_2. Following 24 hours of post-confluence air-liquid interface incubation, a thin film of fluid formed in the apical chamber — termed *in vitro* derived oviduct fluid (*iv*DOF). *iv*DOF was isolated for analysis. The term 'native' is used to describe *iv*DOF resulting from untreated epithelia [31].

2.5. Genistein Transport Studies

BOECs were incubated for 20 minutes at 39°C in 5% CO_2 in a liquid-liquid interface of 4 ml (2 ml apically and basally) normal Krebs Ringer (KR) solution, after which, genistein transport experiments were conducted by supplementing the basal chamber with one of four concentrations of genistein for 150 minutes. Genistein was prepared as a 10 mM stock in dimethyl sulfoxide (DMSO) owing to its limited solubility in ethanol [36]. Final concentrations of genistein supplemented were: 50 µM [10 µl stock in 3990 µl total KR (0.25 % *v/v* vehicle contribution), 100 µM (0.5 % *v/v*), 150 µM (0.75 % *v/v*), or 180 µM (0.9 % *v/v*) (Figure 1).

Krebs Ringer medium was sampled (30 µl) apically and basally at regular intervals. TEER values remained in excess of 700 $\Omega \cdot cm^{-2}$ throughout all experiments conducted. Unless otherwise stated, transport experiments were done at 39ºC with the exception of sampling (~ 30 second duration) being performed at laboratory temperature (~ 21ºC). Medium sampled for analysis was not replaced in order to avoid unnecessary dilution. The apparent permeability coefficient (P_{app}) of genistein transport was also determined and is defined as: $P_{app} = dQ \cdot dt^{-1} \cdot (AC_0)^{-1}$ where dQ/dt is the rate of genistein appearance ($\mu M \cdot min^{-1}$), C_0 is the initial concentration of genistein (µM), and A is the surface area of the monolayer (cm^2). P_{app} is therefore expressed in units of $cm^2 \cdot min^{-1}$ — adapted from [37].

2.6. Genistein Quantification

Samples collected from transport experiments were analyzed individually using an Agilent 1100 HPLC coupled with an Agilent Zorbax™ C-18 silica based column. Genistein eluted from the column with a buffer consisting of 99.5% methanol + 0.5% formic acid and detected at 288 nm. Quantification was performed relative to a standard curve ($R^2 = 0.9996$).

2.7. BOEC Supplementation for *iv*DOF Analyses

Upon reaching confluence BOECs were incubated in an air:liquid interface for 24 hours at 39°C in 5% CO_2 in culture medium basally supplemented with a final concentration of either: 100 μM genistein (in DMSO), 0.1% (*v*/*v*) DMSO, 14.7 pM E2 (in ethanol), or 1% (*v*/*v*) ethanol. The *iv*DOF accumulated following 24 hours of BOEC exposure was subsequently stored at -20ºC until amino acid composition analysis was conducted by HPLC.

2.8. Amino Acid Quantification

HPLC was used to measure 18 amino acids as previously described by Humpherson and coworkers [38]. In brief, amino acids present in *iv*DOF samples were derivatised with orthopthaldialdehyde (OPA) reagent supplemented with 1 mg/ml beta mercaptoethanol (β-ME) forming conjugates which emit fluorescence at 450 nm when excited at 330 nm. Following derivatisation, reverse phase chromatography was performed through an Agilent 1100 HPLC coupled with a Phenomenex HyperClone® 5mm C-18 ODS 250 mm x 4.6 mm (extended) column. Gradient elution used two buffers: (A) 80% 83 mM sodium acetate (pH to 5.9 using glacial acetic acid), 19.5% methanol, 0.5% tetrahydrofuran (THF), and (B) 80% methanol and 20% 83 mM sodium acetate at 30°C for 60 minutes at a flow rate of 1.3 ml/min. Amino acids were separated based on retention time — as detected by fluorescence (absorbance unit peak) and quantified based on peak area relative to known standards [39].

2.9. Caco-2 Culture

As a ‘negative control’ the protocol for isolating fluid was repeated using cell-line derived absorptive colorectal epithelial adenocarcinoma cells (Caco-2). Specifically,

cells from the primarily absorptive HTB-37 human colon carcinoma (Caco-2) line were routinely cultured in T75 polystyrene flasks at 37°C in 5% CO_2 in 95% air. Culture media comprised 12 ml high glucose DMEM supplemented with 15% foetal calf serum (*v*/*v*), 584 $mg \cdot l^{-1}$ glutamine, 1% minimum non-essential amino acids, 100 $U \cdot ml^{-1}$ PenStrep and 0.25 $\mu g \cdot ml^{-1}$ amphotericin B. Media was renewed every 48 hours until cells reached 90–95% confluence, at which point the cells were extracted with trypsin–EDTA solution (3 ml) from each flask and seeded to Transwell™ membranes at a density of 0.26×10^6 $cells \cdot well^{-1}$. Media was replenished every 48 hours and for 20 days until TEER was in excess of 900 $\Omega \cdot cm^{-2}$ — similarly to [40].

2.10. Statistical analysis

Statistical analyses were performed using Prism Graphpad 6 software for Apple Macintosh. Unless otherwise stated, all statistical analysis was two-way analysis of variance (ANOVA) followed by a Holm-Sidak non-parametric *post hoc* analysis.

3. Results

3.1. Transport Kinetics

At all four concentrations, genistein permeated the *in vitro* bovine oviduct epithelial monolayer in a basal to apical direction in accordance with a broadly polynomial (non-linear) kinetic profile (Figure 2A). More specifically, genistein transport by BOECs was greater than the linear rate of spontaneous diffusion across a blank membrane (negative control) during the first 45 minutes of flux (Figure 2B). Following 60 minutes of transport, the rate of genistein movement across BOECs was the same as that across blank Transwell membranes (Figure 2C). Figure 2 therefore shows that genistein flux at all four concentrations measured occurred by two distinct phases of transport; an initial concentration-dependent *burst* phase (Figure 2B), followed by a slower concentration independent *plateau* phase (Figure 2C).

As a first approach to discover whether the *burst* and/or *plateau* phases of transport observed in Figure 2 were facilitated, the experiment was conducted at laboratory temperature (~21 ºC) in addition to the physiologically relevant incubator temperature of 39 ºC. Figure 3A shows that this temperature reduction significantly reduced genistein flux at 20 minutes. Taking this observation further and plotting the data from Figure 2 as the initial concentration of substrate *vs.* the initial rate of genistein transport (Figure 3B) shows that the data fit both classic facilitated and passive kinetic profiles with respective R^2 values of 0.770 and 0868.

To determine whether there was a difference in the directionality of transport, 100 µM genistein was added to the apical (luminal) chamber and its depletion was measured in comparison to that from the basal to apical direction (Figure 4A). The total rate of

accumulation of genistein in the respective chambers was also measured (Figure 4B) and is presented as apparent permeability coefficients (P_{app}). These data indicated no difference in genistein flux directionality.

In addition, there was no difference in directionality when genistein was added at equilibrium (Figure 5A). However when this experiment was conducted it became apparent that the total (sum) of genistein present from both chambers decreased over time. Figure 5B (insert) is a condensed re-plot of the concentration of genistein internalised and/or degraded *vs.* time.

3.2. Effects on *iv*DOF

Figure 6 shows that 100 μM genistein supplementation significantly modified the secretion by oviduct epithelia of 12 of the 18 amino acids measured. However the vehicle DMSO supplemented in isolation also influenced the amino acid composition of *iv*DOF relative to native – specifically serine, glutamine, glycine, threonine, arginine, and leucine secretion increased and histidine was decreased.

Figure 6 indicates that genistein does act independently of DMSO, but not as an E2 analogue. Figure 6 moreover confirms that E2 independently alters *iv*DOF composition; with the same figure showing that 0.1% (*v*/*v*) ethanol only marginally impacts the secretion of histidine.

4. Discussion

This study aimed to use a novel *in vitro* model of the bovine oviduct epithelium to investigate the effects of the dietary isoflavone genistein on luminal fluid composition.

4.1. Transport Kinetics

The first research question was (***a***) does genistein traverse the oviduct epithelium? Genistein crossed the oviduct epithelial membrane, and moreover appeared to do so in accordance with a biphasic (*burst* and *plateau* phase) kinetic profile (Figure 2). The initial *burst* phase of genistein flux occurred at a rate significantly higher than that of spontaneous diffusion across a Transwell membrane free of cells (Figure 2B), whereas the subsequent *plateau* phase was not (Figure 2C).

A secondary aim subsequently became to gain some insight into the potential mechanism of apical genistein flux. From an energetic perspective, the fact that genistein initially transverses the oviduct epithelium faster than the rate of diffusion (Figure 2B) indicates that genistein flux into the oviduct lumen *in vitro* could be driven by facilitated diffusion (passive transport) and/or primary active (ATP-dependent) transport.

It is well established that active transport is temperature dependent [41]. Specifically, the rate of active transport decreases as the temperature deviates from physiological [42]. To test whether either phase (*burst* or *plateau*) of genistein flux might be active, the transport experiment was repeated at laboratory temperature (~21 ºC) (Figure 3A). Figure 3A shows that the ~18 ºC temperature reduction impaired genistein movement at 20 minutes, suggesting that the initial *burst phase* observed could be partly actively

246 mediated.

247 In addition, passive transport is kinetically characterized as the positive linear
248 relationship between the initial rate of transport and the initial concentration of
249 substrate, whereas facilitated transport is kinetically characterized as a positive
250 hyperbolic relationship between the initial rate of transport and the initial
251 concentration of substrate. With this in mind, the experimentally determined initial
252 rates of genistein transport relative to the initial concentration of genistein present
253 were plotted (Figure 3B) and revealed that genistein permeation of the *in vitro* oviduct
254 epithelium fitted both passive and facilitated curves with regression coefficients (R^2)
255 of 0.868 and 0.770 respectively.

256 In spite of this, when genistein was added to both apical and basal compartments of
257 the Transwell membranes in equilibrium (Figure 5), there was no transport of
258 genistein against a concentration gradient, and therefore no active movement. Hence
259 the most likely mechanism underlying the initial *burst phase* of genistein flux is
260 facilitated diffusion, with the decreased transport at 20 minutes at 21 ºC perhaps
261 attributable to a decrease in Brownian motion [43] of genistein at 21 ºC and therefore
262 impairing flux.

263 With regard to the *plateau* phase (Figure 2C), transport remaining unaffected by a
264 reduction in temperature (Figure 3) and the rate of flux being comparable to the
265 spontaneous diffusion of genistein, both imply the *plateau* phase of flux occurs by
266 passive diffusion as genistein approaches equilibrium [44].

267 Spatially, three potential routes for genistein transport exist: (***i***) paracellular flux, (***ii***)
268 intracellular movement or (***iii***) a combination of both routes. Paracellular flux is a

passive (ATP-independent) process whilst intracellular flux is generally an active (ATP-dependent) process [45]. Given the lack of evidence for actively mediated flux, we hypothesize that genistein transverses the *in vitro* oviduct paracellularly. An explanation for the facilitated *burst phase* observed could be the bovine oviduct epithelial phospholipid exterior serving as a surface catalyst, thereby accelerating of transport of this small hydrophobic molecule (Figure 1B) until close to equilibrium.

Although genistein has never been shown to cross the oviduct epithelium before, it has been established that genistein transverses other cellular monolayers *in vitro* including rat immortalised small intestinal epithelial (IEC-18) [46], human corneal epithelial cells (HCEC) [46], rat brain microvascular (BBB) endothelial cells [47], and human colorectal carcinoma epithelial (Caco-2) cells [47-50].

Yang and coworkers [47] reported a P_{app} of genistein flux across Caco-2 monolayers of 16.23 x 10^{-6} $cm^2 \cdot sec^{-1}$ which equates to 0.974 x 10^{-3} $cm^2 \cdot min^{-1}$ determined using 20 µM genistein. Assuming linearity between P_{app} and genistein concentration [51] their P_{app} value would be 4.74 x 10^{-3} $cm^2 \cdot min^{-1}$ for 100 µM. The analogous value obtained in BBB cells was 4.03 x 10^{-6} $cm^2 \cdot min^{-1}$ [47]. The findings presented are similar to the value obtained across the oviduct epithelium: 4.43 x 10^{-3} $cm^2 \cdot min^{-1}$ (Figure 4B). From this it can determined that the oviduct epithelium is inherently less permeable to genistein than BBB cells but more so than Caco-2 cells, although these values are all broadly comparable.

The slight differences observed tentatively point to the observation that the permeability to genistein may be proportional to TEER; specifically cellular confluence is established at >500 $\Omega \cdot cm^{-2}$ in BBB cells [47], >700 $\Omega \cdot cm^{-2}$ in bovine oviduct epithelia [31], and >900 $\Omega \cdot cm^{-2}$ in Caco-2 cells [52]. This observation further

supports the notion that genistein flux across cellular monolayers *in vitro* is paracellular.

Regardless of the exact mechanism of transport, these data suggest that gametes and early embryos could be directly exposed to genistein *in vivo*. To evaluate whether genistein might pose indirect effects, the amino acid composition of *iv*DOF from BOECs treated with genistein was investigated.

4.2. Effects of Genistein Supplementation on *iv*DOF

Figure 6 suggests that 100 μM genistein supplementation significantly affected the secretion of 12 of 18 amino acids relative to native fluid. Specifically, serine, glutamine, glycine, threonine, arginine, tyrosine, valine, phenylalanine, isoleucine, leucine, and lysine were elevated in *iv*DOF from genistein treated BOECs relative to native (untreated), whereas histidine was reduced.

However conducting the vehicle control experiment showed that DMSO also has an effect on the amino acid composition of *iv*DOF (Figure 6). The effect of genistein appeared to be independent of DMSO with regard to the secretion of 5 amino acids measured — specifically glutamine, glycine, arginine, leucine, lysine and isoleucine (Figure 5C). It is difficult to explain this pattern since these 5 amino acids do not share common transporters or chemical characteristics. Nonetheless, although it is challenging to evaluate any specific effect that genistein is having on the *in vitro* oviduct beyond that of DMSO, it appears that genistein does alter the amino acid composition of *iv*DOF. The impact of the modifications of concentrations of each of these amino acids on embryo development is yet to be confirmed, however it is likely that there will be an embryo-response to such changes, given the importance of these

316 amino acids in early development. For example, glutamine serves as a critical energy
317 source [53], whereas glycine plays an important osmoregulatory function [54]. In
318 light of this, the aim became to evaluate whether genistein acts on the *in vitro* oviduct
319 epithelium as an E2 analogue.

320 The affinity of genistein for ERα and and ERβ is 0.7% and 13% respectively of that
321 for the endogenous ligand E2 [26]. Although not a high percentage, the corresponding
322 dissociation constants can be calculated to be as low as 7 nM for ERα and 0.6 nM for
323 ERβ. Given these high affinities of genistein for the oestrogen receptors, it is
324 unsurprising that genistein exerts oestrogenic effects by activating oestrogen response
325 element (ERE) regulated genes in several mouse tissues, as determined by ERE-
326 luciferase linked reporter assays [55], activating ERE regulated genes in human breast
327 cancer cells as determined by qRT-PCR mRNA quantification [56], and by means of
328 modulating rat behaviour [57].

329 To evaluate whether genistein may be acting on the *in vitro* oviduct as an E2 mimic,
330 BOECs were treated with E2 at a physiological concentration. Although one might
331 assume that the amino acid profiles of *iv*DOF obtained from genistein and E2 would
332 be similar, it was observed that 7 of the 18 amino acids measured were significantly
333 different. The data therefore suggest that genistein does impact the composition of
334 oviduct secretions *in vitro* but not as an E2 mimic. However given the effects of
335 DMSO, future work is required to confirm this.

336 **4.3. Additional Observations**

337 From a model validation perspective, the fact that apical accumulation of fluid was
338 not detected in Caco-2 cells, an absorptive epithelium grown on Transwell

membranes, provides confidence in the experimental protocol employed in addition to the secretory nature of the oviduct cells used in the *in vitro* oviduct model.

4.4. Summary

Numerous studies have been conducted on the bioavailability of isoflavones in adults [58-62] but little is known about the possible delivery and effects of flavonoid conjugates on specific tissues, including the oviduct. The data presented show that (***a***) the oviduct epithelium is permeable to genistein, and facilitates its transport into the lumen in accordance with a biphasic kinetic profile, and (***b***) that genistein presence impacts the amino acid composition of *iv*DOF.

Within the wider context of biology the results demonstrate the potential use of this *in vitro* model in characterising the transport or barrier properties of the oviduct towards a range of circulating xenobiotics.

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Figure Legends

Figure 1. The molecular structures of **A**: Genistein-7-Glucoside (Genistin), **B**: Genistein, **C**: E2, **D**: Dimethyl Sulfoxide (DMSO), and **E**: Ethanol. **F**: Schematic diagram of the basic experimental premise of investigating the transport kinetics of genistein across the bovine oviduct epithelium at liquid-liquid interface (Krebs Ringer medium) at four concentrations, and **G**: An analogous schematic highlighting the air-liquid interface (culture medium) based experimental premise of investigating the impact of genistein on *iv*DOF composition in the capacity of a E2 mimic in conjunction with associated vehicular controls: DMSO and ethanol.

Figure 2. **A**: The apical accumulation of genistein across the *in vitro* bovine oviduct epithelial monolayer at four concentrations **(**50 µM, 100 µM, 150 µM and 180 µM) over 150 minutes. **B**: Initial *burst phase* genistein flux across BOECs in addition to across empty Transwell membranes [negative control (N)]. **C**: Secondary *plateau phase* genistein transport across BOECs and blank membranes (all n=3 ± SD). Statistically significant differences were determined by 2-way ANOVA coupled with the Holm-Sidak *post hoc* test. * represents $p \leq 0.05$ and **** $p \leq 0.0001$.

Figure 3. A: The apical flux of genistein (100 µM) across the BOEC epithelium at physiological (39 ºC) and lab (21 ºC) temperature (n=3 ± SD). One statistically significant difference was determined by unpaired t-test (p=0.002). **B:** Genistein transport data plotted as the initial rate of transport ($\mu M \cdot min^{-1}$) *vs.* the initial genistein concentration (µM), showing a good fit to both passive ($R^2 = 0.868$) and facilitated ($R^2 = 0.770$) kinetic profiles (n=3).

Figure 4. **A**: The respective accumulation of genistein in each compartment plotted as

concentration (μM) *vs.* time (minutes) (n=4 ± SD). **B**: The corresponding P_{app} values of the bovine oviduct epithelium to genistein accumulation was 4.43 x 10^{-3} $cm^2 \cdot min^{-1}$ (basal to apical; n=7 ± SEM) and 4.26 x 10^{-3} $cm^2 \cdot min^{-1}$ (apical to basal; n=7 ± SEM) with a genistein concentration of 100 μM.

Figure 5. **A**: The concentration of apical and basal genistein *vs.* time (minutes) when added in equilibrium at 50 $\mu M \cdot chamber^{-1}$. **B**: The depletion of total genistein (all n=3 ± SD).

Figure 6. The amino acid composition of *iv*DOF accumulated apically from native (untreated) BOECs (n=3 ± SD) (black) *vs* that from BOECs basally treated with 100 μM genistein (n=6 ± SD) (grey) *vs iv*DOF derived from oviduct epithelia exposed to 14.7 pM 17β-oestradiol (n=3 ± SD) (red) *vs iv*DOF derived from BOECs supplemented with 1% *v*/*v* dimethyl sulfoxide (DMSO) - the genistein vehicle control (n=3 ± SD) (green) *vs iv*DOF derived from BOECs supplemented with 0.1% *v*/*v* ethanol – the 17β-oestradiol vehicle control (n=3 ± SD). *iv*DOF accumulated over 24 hours. Statistically significant differences were determined by two-way ANOVA followed by a Holm-Sidak *post hoc* analysis, where *a* represents $p \leq 0.0001$, *b* represents $p \leq 0.001$, *c* represents $p \leq 0.01$, and *d* represents $p \leq 0.05$.

Figures

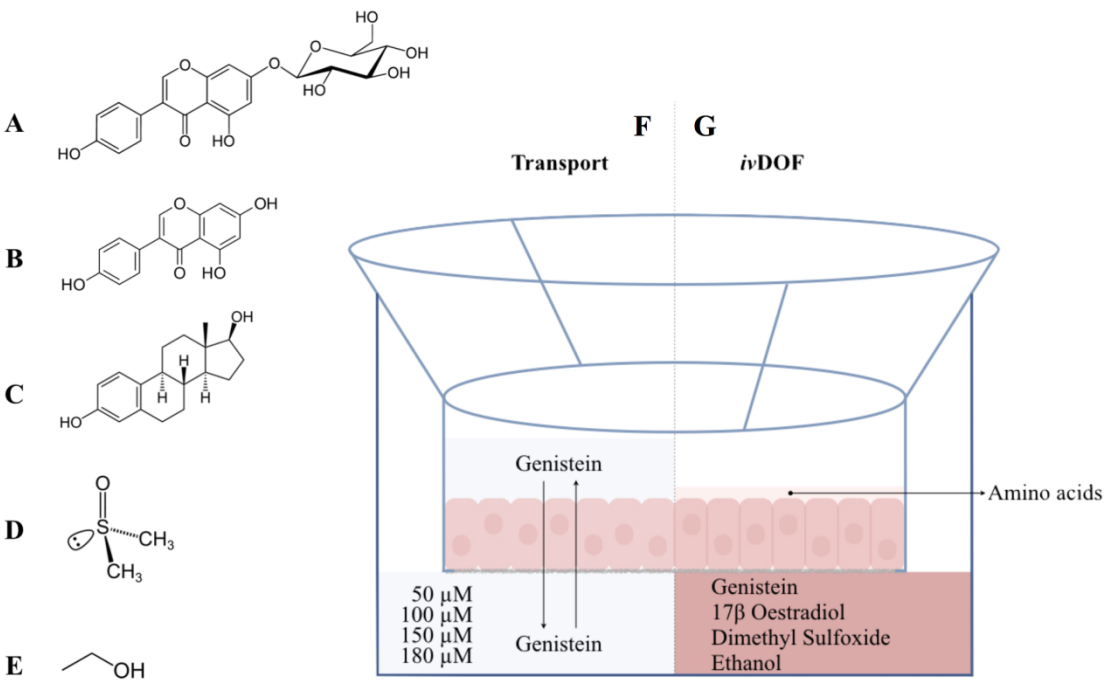
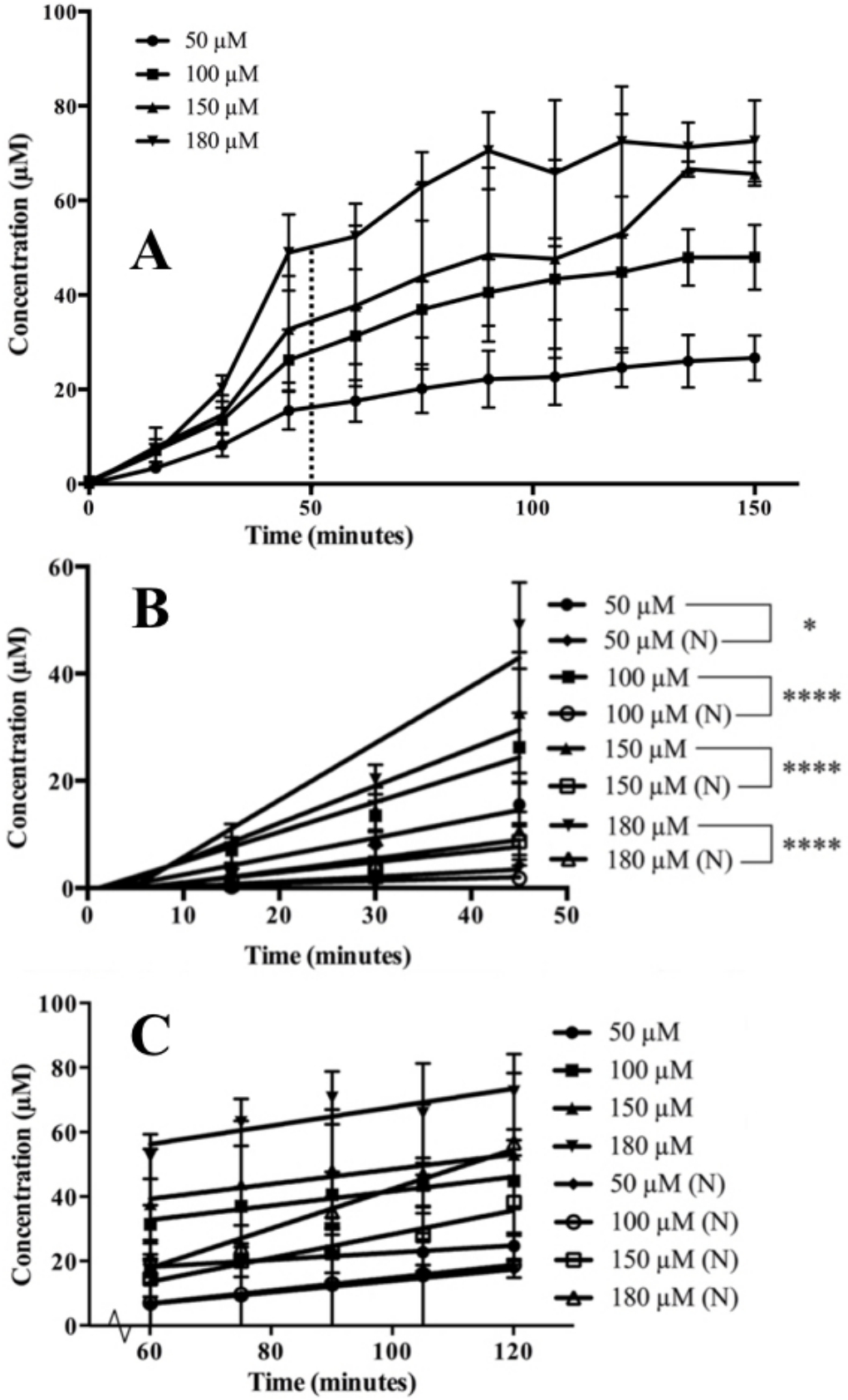


Figure 1



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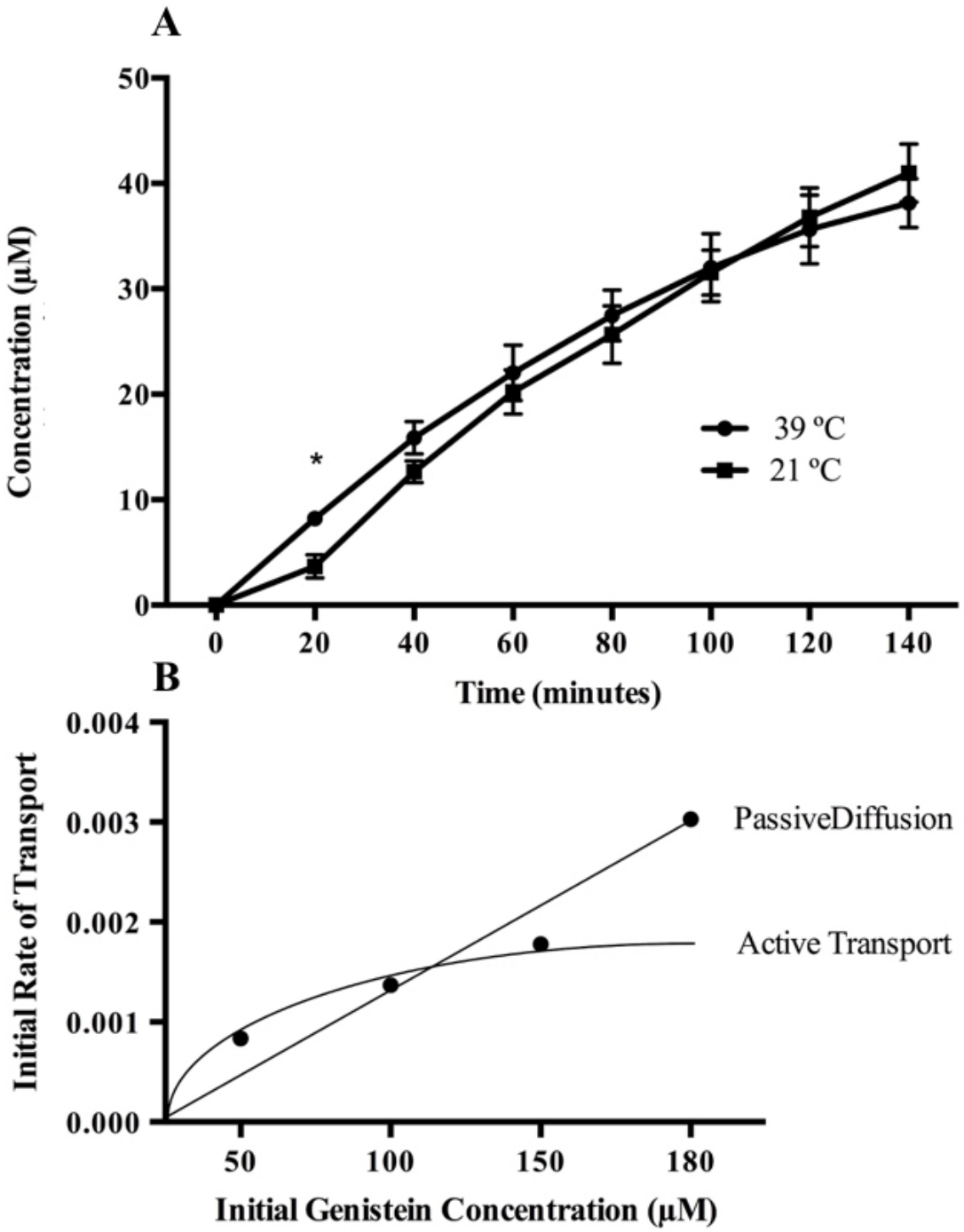

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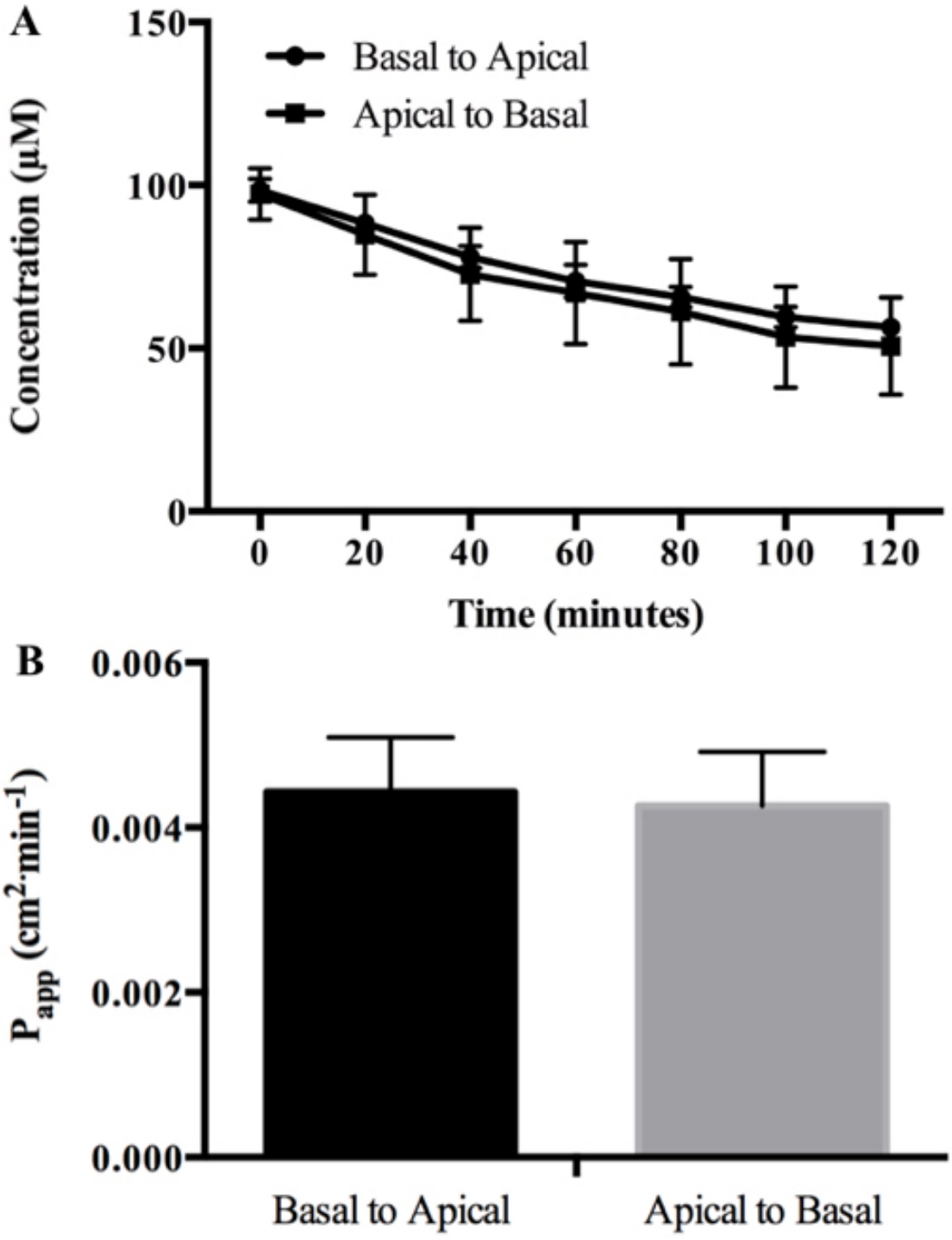

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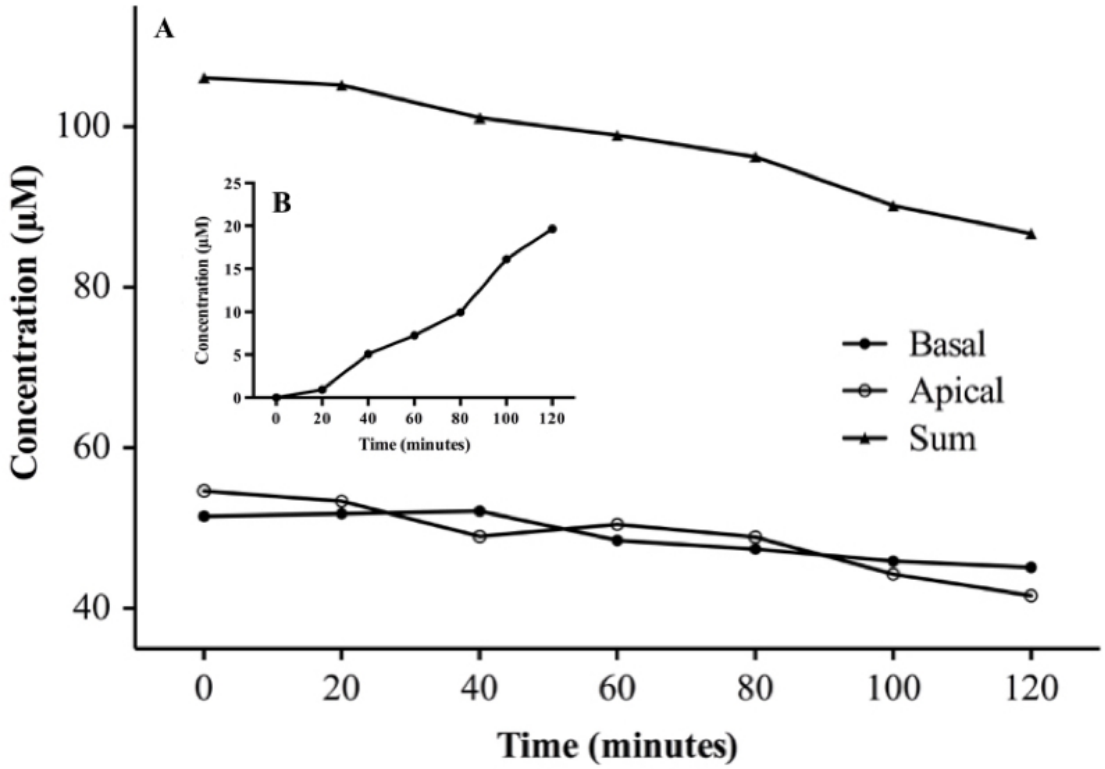

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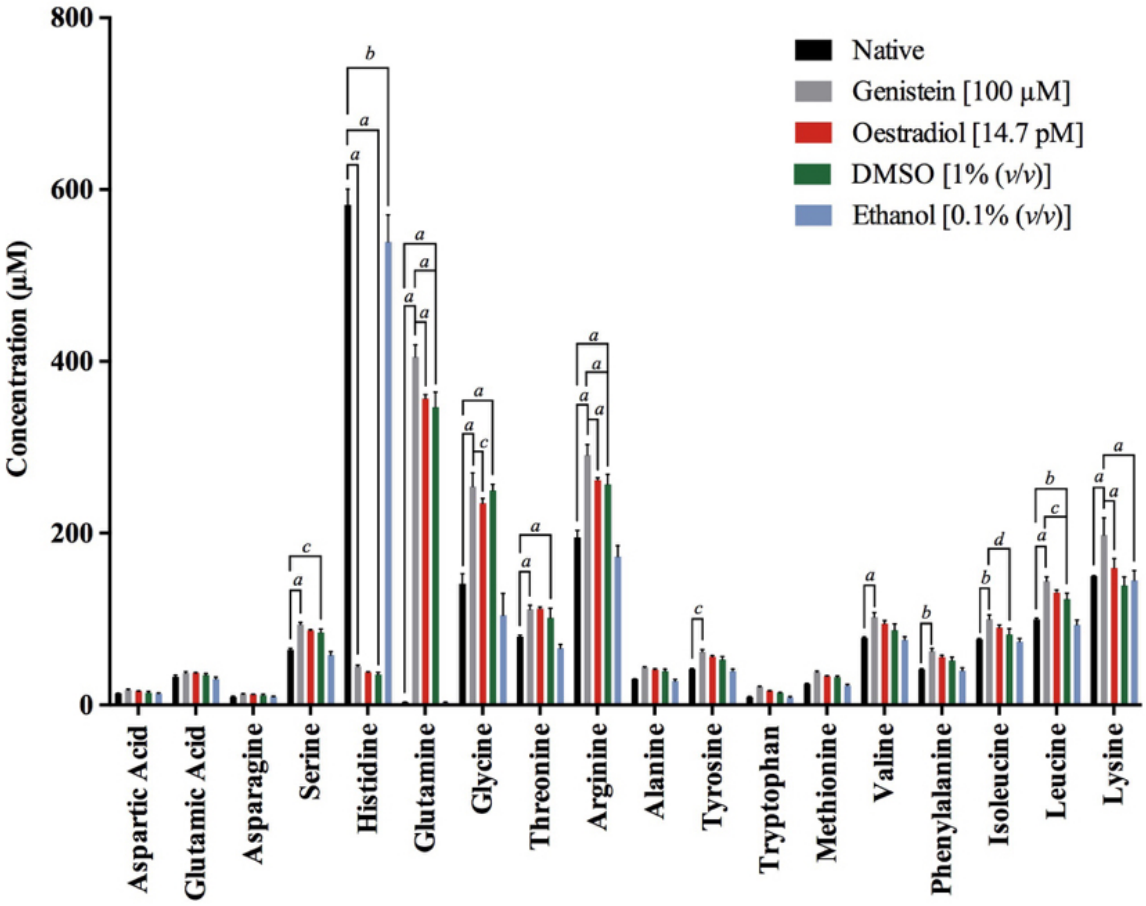

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617 **Figure 6**

Abstract

The dietary derived isoflavone and oestrogen analogue, genistein, is known to perturb fundamental reproductive events such as implantation and embryo cleavage. However the question of whether genistein is able to traverse the oviduct epithelial monolayer and impact oviduct fluid secretion remains unclear. This study tests these research questions using a bioartificial oviduct to show that genistein permeates the oviduct lumen *in vitro* with a biphasic (*burst* and *plateau*) kinetic profile, faster than spontaneous diffusion, and alters the amino acid composition of *in vitro* derived oviduct fluid (*iv*DOF) but not as an oestrogen analogue. In addition to offering insights into the potential mechanisms of these findings, this manuscript demonstrates the potential to use the bioartificial oviduct model to characterise the transport or barrier properties of the oviduct towards a range of circulating xenobiotics.

Highlights

This manuscript utilises an existing technology (bioartificial oviduct) for the novel application of investigating the effects of genistein – a dietary derived isoflavone known to impair reproductive capacity – on the oviduct *in vitro*. The data show that genistein transverses the oviduct epithelium biphasically and moreover impacts the luminal secretion of amino acids. This could explain, in part, previous reports of genistein perturbing central reproductive events such as implantation and cleavage.