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Genistein crosses the bioartificial oviduct and alters secretion composition

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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.

1. Introduction

Genistein-7-glucoside [G7G (Fig. 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 (Fig. 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 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·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 (Fig. 1B), which resembles 17β-oestradiol (E2) (Fig. 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 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

Abbreviations: *iv*DOF, *in vitro* derived oviduct fluid; BOEC, bovine oviduct epithelial cell; G7G, genistein 7-glucoside.

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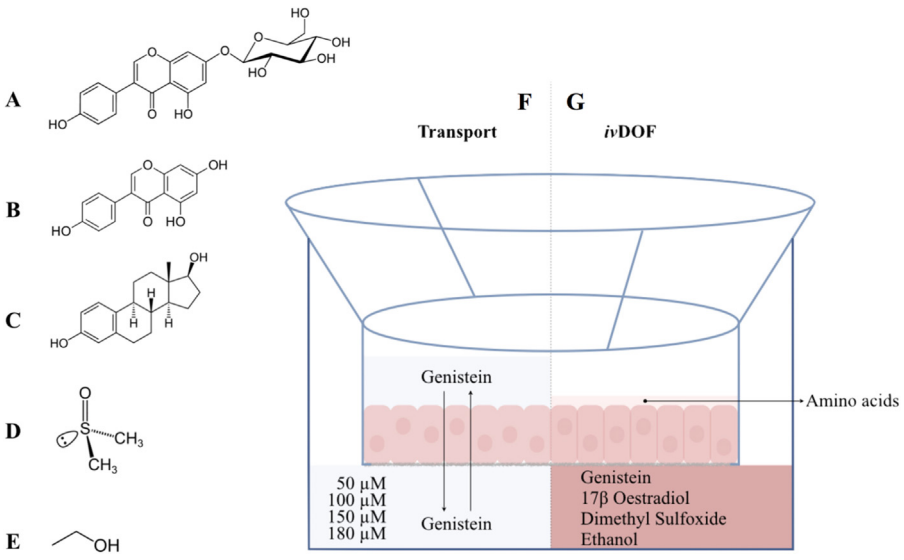

Fig. 1. The molecular structures of A: Genistein-7-Glucoside (Genistin), B: Genistein, C: E2, D: Dimethyl Sulfoxide (DMSO), and E: Ethanol. F: Schematic diagram of the basic experimental premise of investigating the transport kinetics of genistein across the bovine oviduct epithelium at liquid-liquid 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.

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 tract has never been confirmed.

Building on these findings, the *in vitro* oviduct model recently established by Simintiras and coworkers [31] has been used to investigate whether effects of dietary genistein on the developing conceptus may be direct or indirect. The research questions of this study therefore are: (***a***) does genistein traverse the oviduct epithelium, thereby permitting a direct effect on the embryo? (***b***) Does genistein supplementation impact the amino acid composition of *in vitro* derived oviduct fluid (*iv*DOF), thus potentially affecting the embryo indirectly? (***c***) If so, is the amino acid 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 min 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 min at 400 × *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 min at 350 × *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 h 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 1×10^6 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 h 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