DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



# UDP-glucuronosyltransferase (Ugt) 1a enzymes are present and active in the mouse blastocyst.

**Abby C. Collier, Yasuhiro Yamauchi, Brittany L.M. Sato, Luc R.A. Rougée, and Monika A. Ward**

Faculty of Pharmaceutical Sciences, University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC V6T 1Z3, CANADA (*ACC*)

Institute for Biogenesis Research, John A. Burns School of Medicine, University of Hawaii, 1960 East-West Rd, Honolulu, HI 96822, USA. (*YY, MAW*)

Natural Sciences and Mathematics, Chaminade University of Honolulu, 3140 Waialae Avenue, Honolulu, HI 96816, USA (*BLMS*)

Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii, 651 Ilalo St, Honolulu, HI 96813, USA. (*ACC, BLMS, LRAR*)

DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



**Running title:** UGT in mouse blastocysts

* Corresponding author:
Abby C Collier,
Pharmaceutical Sciences,
6609-2405 Wesbrook Mall,
Vancouver, BC V6T 1Z3.
Tel. (604) 827-2380,
E-mail: **abby.collier@ubc.ca**

**Number of text pages (not including title page, including references):** 12
**Number of Tables:** 0
**Number of Figures:** 3
**Number of words in abstract**: 192
**Number of words in Introduction**: 432
**Number of words in Discussion**: 680
**Number of References:** 31

**Non-standard abbreviations:** BD62F1: hybrid mouse from C57/black6 crossed with DBA/2 mouse; CZB medium: Chatot, Ziomek and Bavister's medium; DAPI: 4′,6′-diamidino-2-phenylindole; ECL: electrochemiluminescence; HNF: hepatic nuclear factor; ICSI: intra-cellular sperm injection; IVF: in vitro fertilization; PPARδ: peroxisome proliferator-activated receptor, subtype delta; RXR: retinoic-X-receptor; Ugt: UDP-glucuronosyltransferase gene and protein (mouse); UGT: UDP-glucuronosyltransferase gene and protein (human)

**Abstract**

The UDP-glucuronosyl transferase enzymes (UGT) are critical for regulating nutrients, hormones and endobiotics, and for detoxifying xenobiotics. Human and murine fetuses are known to express glucuronidation enzymes, but there are currently no data prior to implantation. Here we addressed this gap in knowledge and tested whether Ugt enzymes are already present in preimplantation stage embryos. Blastocysts were obtained after in vitro fertilization with gametes from B6D2F1 hybrid mice, and embryo culture. Protein expression and localization were determined using pan-specific UGT1A and UGT2B, as well as anti-human isoform-specific antibodies. Immunofluorescence analysis showed that blastocysts expressed Ugt1a globally, in the cytoplasm and nuclei of all the cells. Western blots demonstrated the presence of Ugt1a6 but not Ugt1a1, 1a3, 1a4, or 1a9. The Ugt2b proteins were not detected by either assay. The level of Ugt activity in murine blastocysts was comparable to that of the adult human liver (per-milligram-of-protein), but the activity of β-glucuronidase, an Ugt-partnering enzyme responsible for substrate regeneration, was lower. Altogether, these data confirm that Ugt1a proteins are present and active in preimplantation murine embryos and point to a potential role for these proteins in implantation and early embryonic and fetal development.

DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



**Introduction**

The UDP-glucuronosyl transferases (UGTs) are a superfamily of enzymes that catalyze the conjugation of glucuronic acid to molecules primarily to facilitate systemic elimination (Radominska-Pandya et al., 1999). These enzymes are critical for eliminating chemicals, steroid hormones, nutrients and other endobiotics, thereby regulating systemic levels of compounds and maintain homeostasis.

The first evidence for Ugts in mouse fetal development was in 1975 when Fyffe and Dutton reported on development of glucuronidation in murine tissues (Fyffe and Dutton, 1975). Shortly after this, it was discovered that in humans the activities of hepatic UGTs (the primary site of metabolism) do not develop until close to term and/or after birth (Onishi et al., 1979; Kawade and Onishi, 1981). Hence, during gestation, glucuronidation is performed largely by the maternal liver and placenta (Collier et al., 2002a; Collier et al., 2002b). Since the placenta is of fetal not maternal origin, yet expresses active UGTs while the fetus does not, genetic, tissue-specific and environmental factors have all been inferred to influence UGT in development.

We have been working towards elucidating the roles of UGT/Ugt in reproduction, gestation and development. In the mouse, procedures commonly used in assisted reproduction such as in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and embryo biopsy, can dysregulate placental function and fetal development (Collier et al., 2009; Collier et al., 2012; Sugawara et al., 2012). These effects occur, in part, through interference with Ugt expression and function in murine placental and fetal tissues (Collier et al., 2009; Raunig et al., 2011a; Raunig et al., 2011b; Collier et al., 2012). Since these studies were performed in term tissues, a question remained as to when Ugt enzymes arise in mouse embryonic and fetal tissues. We hypothesized that Ugt expression and activity may occur as early as the blastocyst stage of embryonic development, when the precursors of the placenta begin to differentiate.

DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



The mouse model offers the opportunity to study early developmental stages such as blastocysts and cleavage-stage embryos in culture, and researchers can perform manipulations that may not or cannot be attempted with human embryos. Thus, mouse models of assisted reproduction and development are common, particularly in the developmental and toxicological fields. Recently, mRNA microarray analysis demonstrated that in the murine maternal liver, kidney, small intestine, and placenta Ugt transcripts were stable across gestation in normal pregnancy (Shuster et al., 2013). The authors inferred that the pregnancy process itself did not substantially alter transcript levels of Ugt genes. Expanding and complementing these studies, here we show expression and activity of Ugt1a enzymes in preimplantation murine embryos, providing the earliest evidence for active Ugts in mammalian development.



## Materials and Methods

*Animals*

Mice B6D2F1 (C57BL/6 x DBA/2) were obtained from the National Cancer Institute (Raleigh, NC) at 6-8 weeks of age. Mice were fed *ad libitum* with a standard diet and maintained in a temperature and light-controlled room (22˚C, 14 h light/10 h dark), in accordance with the guidelines presented by the National Research Council (USA). The protocol for animal handling and treatment procedures was reviewed and approved by the Animal Care and Use Committee at the University of Hawaii.

Mice were used as oocyte and sperm donors for in vitro fertilization (IVF), performed as reported by Sugawara et al., (2012). Briefly, oocytes were collected from females induced to superovulate and epididymal sperm were collected from males by release from caudae epididymides directly into T6 medium and capacitated for 1.5 h at 37ºC in a humidified atmosphere of 5% $CO_2$. Gametes were co-incubated for 4 h, then oocytes were washed with HEPES-CZB followed by at least one wash with CZB medium. Fertilized oocytes with two well-developed pronuclei and

DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



extruded second polar body were cultured in 50 μL drops of CZB for 120 h, when they reached expanded blastocyst stage and were used for experimentation.

*Ugt protein expression and localization in situ (ICC) with fluorescence confocal microscopy*

Immunofluorescence analysis of blastocysts was performed as previously described (Alarcon, 2010). The primary antibodies and concentrations were: pan-specific UGT1A and UGT2B (1:200; Santa Cruz Biotechnology, CA, sc-25847 and sc-23479). Secondary antibodies were Biotin-SP-conjugated AffiniPure Donkey Anti-rabbit IgG for UGT1A and Donkey Anti-Goat IgG for UGT2B (1:200 both; Jackson ImmunoResearch Labs Inc. PA). Streptavidin-fluorescein RPN 1232 (Amersham Biosciences) was used to bind to biotin, then stained embryos mounted on slides in Vectashield with 4′,6′-diamidino-2-phenylindole (DAPI, Vector Laboratories, CA). Embryos were analyzed with an FV1000-IX81 confocal fluorescent microscope with MetaMorph Microscopy Automation & Image Analysis Software and Fluoview v. 2.1 software (Olympus, PA). The same settings (exposure time, pinhole size, and gain) were used to record images of embryos that were stained in the same batch and used for comparison of fluorescence intensities . Fluorescence intensities of embryos were compared with Image J (http://imagej.nih.gov/ij/).

*Ugt protein expression (western blot) in mouse blastocysts*

Western blotting was performed for Ugt protein expression analysis as previously described (Miyagi and Collier, 2011), using 20 μg pooled blastocyst protein in each well. Antibody incubations were: UGT1A and UGT2B, 1:1,000; and UGT1A1 & UGT1A6, sc-27415 and sc-27434, 1:2,000, (Santa Cruz Biotechnologies, CA), UGT1A3 (mouse-anti-human Abcam.com, ab574000) and UGT1A4 and UGT1A9 (sheep-anti-human, gifts from Prof. MWH Coughtrie, U British Columbia, Canada). The UGT antibodies used were all to human proteins. UGT1A and 2B have been demonstrated to cross-react with mouse proteins, but the specificity of the

DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



UGT1A6 antibody for Ugt1a6 has not been confirmed in the mouse. Secondary antibody were donkey-anti-species biotin, 1:8,000 (Jackson Immunolabs, Westgrove, PA) and biotin detection was done with streptavidin-HRP-biotin (1:10,000, GE Healthcare, Piscataway, NJ). Detection was done with ECL plus (GE Healthcare). Proteins were sized by comparison to a Rainbow Marker (GE Healthcare) and recombinant controls. Each membrane contained a positive control human tissue (microsomes derived from 200 pooled human livers, Xenotech Lenexa KS) and recombinant positive control (GE Healthcare). All antibodies were blotted 3 times, and each gel contained 3 individual lanes representing different pools of blastocysts. Exposures for each antibody were performed for the same amount of time, but exposure time between antibodies differed (UGT1A and UGT2B, 45 min; UGT1A1, 1A3, 1A4, 1A9, 20 min; UGT1A6, 90 min).

*Biochemical detection and quantification of UGT and β-glucuronidase activity*

The assay for Ugt activity with 4-methylumbelliferone (4-MU) was carried out as previously described, with 0.5 mg/mL blastocyst protein and alamethicin 50 μg/mg protein as the activator (Collier et al., 2000). The intra- and inter assay CVs for the slopes of the standard curves were 4.1% and 9%, respectively, and the intra-and inter-assay variability for pooled adult human liver microsomes (n = 200, positive control) 12.2% and 14.1%, respectively.

The assay for β-glucuronidase activity was performed with 4-methylumbelliferone glucuronide according to the method of Trubetskoy and Shaw (Trubetskoy and Shaw, 1999). The accuracy and precision of the standard curves were the same as reported above for 4MU activity.

*Statistical Analyses*

Data were analyzed using one-way ANOVA, with Bonferroni's multiple comparison post-hoc analysis. Bars presented are means ± SD or SEM, as indicated in figure legends. Statistical

analyses were performed using Graph Pad Prism 5.0 (Graph Pad, San Diego, CA) with $\propto \leq$ 0.05.

## Results

*Ugt expression and localization in blastocysts - immunofluorescence*

Blastocysts (n = 22-30 total per group, 3 experimental replicates) were examined for Ugt1a and Ugt2b expression using confocal microscopy immunofluorescence after staining with pan-specific antibodies, raised in humans for UGT1A and 2B, that have previously been demonstrated to cross-react with mouse isoforms. Proteins from the Ugt1a family were present throughout the blastocysts, with both nuclear and cytoplasmic localization (Fig. 1A). Residual staining for Ugt2b proteins could be observed (Fig. 1A) but the quantitative analysis of fluorescence intensity revealed no differences from background control (Fig. 1B). All examined groups showed similar levels of fluorescence intensity after DAPI nuclear staining, demonstrating comparable cell numbers in the blastocysts (Fig. 1B).

*Ugt expression and localization in blastocysts - western blot*

Western blot for the general Ugt subfamilies demonstrated that murine Ugt1a proteins were present and cross-reactive to UGT1A antibodies (Fig. 2A). Cross immuno-reactivity to anti-human UGT1A1, 1A3, 1A4, and 1A9 antibodies was not observed (Fig. 2B-D, 2F). Proteins reactive to a human UGT1A6 antibody were observed (Fig. 2E). We do not know whether murine Ugt1a6a and/or 1a6b were detected, since Ugt1a6 protein appeared as a doublet (Fig. 2E). It is not uncommon for UGT/Ugt proteins to blot as doublets, and it has been speculated that this is due to dimerization of proteins to form an active unit (Meech and Mackenzie, 1997) and/or antibodies detecting both ER-anchored proteins and cytosolic proteins that are being chaperoned to the ER and have not yet cleaved their homing sequence (Radominska-Pandya et

al., 1999)**.** Cross reactivity to UGT2B antibodies was not detected, (Fig. 2G). Hence, UGT2B/Ugt2b isoform-specific antibodies were not used further.

*Ugt and β-Glucuronidase activities*

Blastocysts showed similar levels of Ugt activity towards the general substrate 4-MU (per milligram of protein basis) as the pooled adult human liver positive control. (Fig. 3A, $P < 0.001$ vs. negative control). Although 4-MU is metabolized by multiple UGT/Ugt isoforms from both the 1A/1a and 2B/2b families (Uchaipichat et al., 2004), UGT1A4 does not metabolize 4-MU. This is, however, moot for the mouse where Ugt1a4 is a pseudogene. Additionally UGT1A1 and UGT1A6 are the most active towards 4-MU, with rates up to 10 times higher than UGT2B and other UGT1A isoforms (Jin et al., 1993; Green et al., 1994; Burchell et al., 2005). Therefore, because neither Ugt1a1, Ugt1a9 nor any Ugt2b proteins were detected, it is plausible that the activity measured is primarily Ugt1a6, although contributions from Ugt1a1, 1a2, 1a5, 1a7c, 1a8, 1a9 and 1a10 cannot be ruled out. For β-glucuronidase, while activities were significantly (Fig. 3B, $P < 0.01$) above background, they were also significantly lower than adult human liver ($P < 0.001$).

## Discussion and Conclusions

Here we demonstrate that Ugt1a but not Ugt2b proteins are expressed in murine preimplantation embryos, with potential positive identification of Ugt1a6. Activity towards the general substrate 4-MU was observed, and was as high as the human liver (on a per milligram of protein basis). This is the earliest that UGT proteins have been found in mammalian development.

There are currently four recognized mammalian families of UGT – UGT1, 2, 3 and 8. While the UGT1A genes share homology between humans and mice (i.e. the human UGT1A1 gene is homologous to mouse *Ugt1a1*) the UGT2B/Ugt2b isoforms are non-orthologous (Mackenzie et

al., 2005). The lack of homology in UGT2B/*Ugt2b* genes does not explain our inability to detect UGT2B proteins using the pan-specific antibody, as it is raised to a consensus sequence and has been used successfully in mice, humans, rats and dogs (Muller et al., 2008). The UGT2A/Ugt2a, 3A/3a and 8A/8a families appear to be homologous between humans and mice, but we did not probe for them due to their restricted (UGT2A/2a, olfaction) and undefined (UGT3, UGT8) roles.

The different subfamilies of UGT/Ugt enzymes show diverse expression and activity profiles. The liver, gastrointestinal tract, and kidney are the major sites of UGT1A/1a enzyme expression and activities in humans and mice, which are primarily responsible for glucuronidation of essential endobiotics (e.g. bile acids, bilirubin), dietary substances and chemicals, with smaller roles in steroid hormone metabolism (Radominska-Pandya et al., 1999). In contrast, while human and rodent UGT2B/Ugt2b members are expressed in the digestive tract, they tend to play more critical roles in steroid-target tissues (Radominska-Pandya et al., 1999). Hence, our failure to find Ugt2b proteins in the blastocysts is surprising because of the association of steroids with establishment of pregnancy and embryogenesis. However, our findings in the mouse show a similar pattern as in humans, where UGT1A6 is the earliest of the UGT isoforms to become present and active in the fetus, while UGT1A1 does not develop until after birth (Onishi et al., 1979; Kawade and Onishi, 1981; Burchell et al., 1989; Miyagi and Collier, 2011). We cannot definitively state that Ugt1a6 is the sole Ugt1a present in murine preimplantation blastocysts because we cannot confirm that the human UGT1A6 antibody is specific for murine Ugt1a6. Despite this, the lack of cross reactivity to anti-human UGT1A1, 1A3, 1A4 and 1A9 makes our case more compelling. We did not expect to observe reactivity to human UGT1A3 or 1A4 antibodies as these are pseudogenes in the mouse, so absence of reactivity to these antibodies is not surprising. Additionally, we did not probe for Ugt1a2, 1a5, 1a7c, 1a8 or 1a10 hence these isoforms may also be present.

Prior to this study, the earliest that UGT enzymes had been reported in development was the presence of UGT immunoreactive cells in human liver parenchyme 32 days post-ovulation and in nucleated embryonic red blood cells 47 days post-ovulation (Hume et al., 1996). Such early expression caused speculation that UGTs may play a role in human embryogenesis. This was further confirmed when the obligate co-factor transporter for UGTs (the UDPGA transporter) was shown to be encoded on the fringe connection, *frc*, gene and involved in the Wingless, Hedgehog, fibroblast and Notch signaling pathways in mice (Goto et al., 2001; Selva et al., 2001). Others have also demonstrated that the nuclear receptors RXR (Königsdorf et al., 2012), PPARδ (Kang et al., 2011) and HNF4 (Duncan et al., 1994) are present in preimplantation murine embryos. Speculatively, the actions of these receptors could be driving Ugt expression in mouse blastocyst, as Ugt/UGTs are responsive to these receptors.

Our data imply a role for Ugt proteins in early murine development and may provide insights into preimplantation conditions in the human. Since mice are the most common laboratory model for pregnancy, and are entrenched in the pre-clinical safety and reproductive toxicology battery (Anon, 1999), better understanding of murine pregnancy, including metabolizing enzymes such as Ugts, has direct benefit. The presence of Ugts at the preimplantation stage of development indicates an as-yet unidentified role of these proteins in mammalian embryonic development that may be critical for good pregnancy outcomes.

***Acknowledgement***

We acknowledge Prof. MWH Coughtrie, University of British Columbia, Canada, for the gifts of sheep-anti-human UGT1A4 and UGT1A9 antibodies.

***Authorship Contributions:***

***Participated in research design*:** Ward and Collier

***Conducted Experiments:*** Yamauchi, Sato, Rougée

***Performed Data Analysis*:** Collier, Yamauchi, Sato, Rougée, Ward

***Wrote or contributed to writing the manuscript:*** Collier, Yamauchi, Sato, Rougée, Ward

## References

Alarcon VB (2010) Cell polarity regulator PARD6B is essential for trophectoderm formation in the preimplantation mouse embryo. *Biol Reprod* 83**:**347-358.

Anon (1999) International conference on harmonisation; guidance on the duration of chronic toxicity testing in animals (rodent and nonrodent toxicity testing); availability. Department of Health and Human Services, Food and Drug Administration, Baltimore MD.

Burchell B, Coughtrie M, Jackson M, Harding D, Fournel-Gigleux S, Leakey J and Hume R (1989) Development of human liver UDP-glucuronosyltransferases. *Devel Pharmacol Therapeut* 13**:**70-77.

Burchell B, Lockley D, Staines A, Uesawa Y and Coughtrie M (2005) Substrate specificity of human hepatic UDP-glucuronosyl transferases. *Meth Enzymol* 400**:**46-57.

Collier A, Ganley N, Tingle M, Blumenstein M, Marvin K, Paxton J, Mitchell M and Keelan J (2002a) UDP-glucuronosyltransferase activity, expression and cellular localization in human placenta at term. *Biochem Pharmacol* 63**:**409-419.

Collier A, Milam K, Rougée L, Sugawara A, Yamauchi Y and Ward M (2012) Upregulation of Ugt1a genes in placentas and fetal livers in a murine model of assisted reproduction. *Placenta* 33**:**77-80.

Collier A, Miyagi S, Yamauchi Y and Ward M (2009) Assisted reproduction technologies impair placental steroid metabolism. *J Steroid Biochem Mol Biol* 116**:**21-28.

Collier A, Tingle M, Keelan J, Paxton J and Mitchell M (2000) A highly sensitive fluorescent microplate method for the determination of UDP-glucuronosyl transferase activity in tissues and placental cell lines. *Drug Metab Dispos* 28**:**1184-1186.

Collier A, Tingle M, Paxton J, Mitchell M and Keelan J (2002b) Metabolizing enzyme localization and activities in the first trimester human placenta: the effect of maternal and gestational age, smoking and alcohol consumption. *Hum Reprod* 17**:**2564-2572.

Duncan S, Manova K, Chen W, Hoodless P, Weinstein D, Bachvarova R and Darnell J Jr (1994) Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. *Proc Natl Acad Sci U S A*. 91:7598-602.

Fyffe J and Dutton G (1975) Induction of UDPglucose dehydrogenase during development, organ culture, and exposure to phenobarbital. Its relation to levels of UDPglucuronic acid and overall glucuronidation in chicken and mouse. *Biochim Biophys Acta* 10**:**41-49.

Goto S, Taniguchi M, Muraoka M, Toyoda H, Sado Y, Kawakita M and Hayashi S (2001) UDP-sugar transporter implicated in glycosylation and processing of Notch. *Nat Cell Biol* 3**:**816-822.

Green M, Oturu E and Tephly T (1994) Stable expression of a human liver UDP-glucuronosyltransferase (UGT2B15) with activity toward steroid and xenobiotic substrates. *Drug Metab Dispos* 22**:**799–805.

Hume R, Burchell A, Allan B, Wolf C, Kelly R, Hallas A and Burchell B (1996) The ontogeny of key endoplasmic reticulum proteins in human embryonic and fetal red blood cells. *Blood* 87**:**762-770.

Jin C-J, Miners J, Lillywhite K and Mackenzie P (1993) cDNA cloning and expression of two new members of the human liver UDP glucuronosyltransferase 2B subfamily. *Biochem Biophys Res Comm* 194**:**496–503.

Kang H, Hwang S, Yoon J, Jun J, Lim H, Yoon T and Song H (2011) Activation of peroxisome proliferators-activated receptor δ (PPARδ) promotes blastocyst hatching in mice. *Mol Hum Reprod.* 17:653-60.

Kawade N and Onishi S (1981) The prenatal and postnatal development of UDP-glucuronyltransferase activity towards bilirubin and the effect of premature birth on this activity in the human liver. Biochem J 198:257-60

Königsdorf C, Navarrete Santos A, Schmidt J, Fischer S and Fischer B (2012) Expression profile of fatty acid metabolism genes in preimplantation blastocysts of obese and non-obese mice. *Obes Facts* 5:575-86.

Mackenzie P, Bock K, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners J, Owens I and Nebert D (2005) Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genom* 15**:**677-685.

Meech R and Mackenzie, P (1997) UDP-glucuronosyltransferase, the role of the amino terminus in dimerization. *J Biol Chem* 272**:**26913-26917.

Miyagi S and Collier A (2011) The Development of UDP-Glucuronosyltransferases 1A1 and 1A6 in the Pediatric Liver. *Drug Metab Dispos* 39**:**912-919.

Muller A, Glattard E, Taleb O, Kemmel V, Laux A, Miehe M, Delalande F, Roussel G, Van Dorsselaer A, Metz-Boutigue M, Aunis D and Goumon Y (2008) Endogenous morphine in SH-SY5Y cells and the mouse cerebellum. *PLoS One* 3**:**e1641.

Onishi S, Kawade N, Itoh S, Isobe K and Sugiyama S (1979) Postnatal Development of Uridine Diphosphate Glucuronyltransferase Activity towards Bilirubin and 2-Aminophenol in Human Liver. *Biochem J* 184**:**705-707.

Radominska-Pandya A, Czernik P, Little J, Battaglia E and Mackenzie P (1999) Structural and Functional Studies of UDP-glucuronosyl transferases. *Drug Metab Rev* 31**:**817-899.

Raunig J, Yamauchi Y, Ward M and Collier A (2011a) Assisted Reproduction Technologies Alter Steroid Delivery to the Mouse Fetus During Pregnancy. *J Steroid Biochem Mol Biol* 126**:**26-34.

Raunig J, Yamauchi Y, Ward M and Collier A (2011b) Placental inflammation and oxidative stress in the mouse model of assisted reproduction. *Placenta* 32**:**852-858.

Selva E, Hong K, Baeg G, Beverley S, Turco S, Perrimon N and Häcker U (2001) Dual role of the fringe connection gene in both heparan sulphate and fringe-dependent signalling events. *Nat Cell Biol* 3**:**809-815.

Shuster D, Bammler T, Beyer R, Macdonald J, Tsai J, Farin F, Hebert M, Thummel K and Mao Q (2013) Gestational age-dependent changes in gene expression of metabolic enzymes and transporters in pregnant mice. *Drug Metab Dispos* 41**:**682.

Sugawara A, Sato B, Bal E, Collier A and Ward M (2012) Blastomere removal from cleavage-stage mouse embryos alters steroid metabolism during pregnancy. *Biol Reprod* 87**:**4, 1-9.

Trubetskoy O and Shaw P (1999) A fluorescent assay amenable to measuring production of beta-D-glucuronides produced from recombinant UDP-glycosyl transferase enzymes. *Drug Metab Dispos* 27**:**555-557.

Uchaipichat V, Mackenzie P, Guo X, Gardner-Stephen D, Galetin A, Houston J and Miners J (2004) Human udp-glucuronosyltransferases: isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. *Drug Metab Dispos* 32:413-23

## Footnotes

This work was funded by the National Institutes of Health [Grants: MD007601, GM103457 and HD072380], and also a Hawaii Community Foundation Grant. Funding bodies had no input into study design, interpretation or decision to publish.

DMD Fast Forward. Published on September 8, 2014 as DOI: 10.1124/dmd.114.059766
This article has not been copyedited and formatted. The final version may differ from this version.



## Figure Legends

**Figure 1: Confocal immunofluorescence analysis of Ugt expression and localization in blastocysts**. **1A:** Exemplary images of blastocysts stained with pan-specific antibodies against Ugt1a and Ugt2b, with DAPI staining to show the cell nuclei. Strong Ugt1a signal is present throughout the blastocysts while Ugt2b shows only weak cytoplasmic staining. No signal is observed in negative (no antibody) control. Scale = 10 µm. **1B:** Quantitative analysis of fluorescence intensity. Levels of DAPI and antibody staining were measured in individual blastocysts. Three replicates were performed with n = 6-11 embryos per group in each triplicate. The Ugt1a and Ugt2b controls are embryos from the same pool as the antibody stained embryos, but for which the antibody was omitted. The graphs show plotted data for individual blastocysts. There were no differences in average DAPI fluorescence intensity between Ugt1a and UGT2b and their respective controls, Ugt1a and Ugt2b, and two sets of controls (P = NS). Ugt1a staining was significantly higher than all other groups ($P < 0.0001$) and no differences were observed between Ugt2b and its control and between two controls (P = NS). Significance was defined by ANOVA with Bonferroni post-hoc comparison. All scatter dot plots show the mean and SEM.

**Figure 2: Detection of Ugt proteins in mouse blastocysts. 2A:** Proteins of the Ugt1a family were present as detected by a UGT1A/Ugt1a pan-specific antibody (55kDa). **2B:** Murine Ugt1a1 was not detected (52 kDa). **2C:** Murine Ugt1a3 was not detected (37 kDa). **2D:** Murine Ugt1a4 was not detected (60 kDa). **2E:** Murine Ugt1a6 was detected (61 kDa) **2F:** Murine Ugt1a9 was not detected (52 kDa). **2G:** Proteins of the UGT2b family were not detected using UGT2B pan-specific antibody (55 kDa). L = human liver positive control. R = recombinant protein positive control, which was a human recombinant the same as the antibody except UGT1A where recombinant UGT1A1 was used and UGT2B where recombinant UGT2B7 was used. Lanes 1, 2,

3 represent 3 different pools of blastocyst protein lysates. Blots were performed 3 times for each antibody and representative blots are shown.

**Figure 3: Total Ugt activity measured using 4-methylumbelliferone (4-MU), and activity of β-glucuronidase in blastocyst lysates. 3A:** Blastocyst lysates (BLAST) demonstrate measurable Ugt activity that was significantly higher than negative control NEG CON A (boiled blastocysts, $P < 0.001$) and NEG CON B (reaction with no cofactor, $P < 0.001$). Ugt activities in blastocysts were not significantly different (NS, $P = 0.91$) than those of the human liver positive control (POS CON). **3B:** Blastocysts (BLAST) show significantly higher β-glucuronidase activity than the negative control (NEG CON, sacchrolactone 5mM, $P < 0.01$), but significantly less than human liver microsomes positive control (POS CON, $P < 0.001$). Significance was defined by ANOVA with Bonferroni's post hoc comparisons as indicated by bars ** = $P < 0.01$, *** = $P < 0.001$. All bars are means ± SD.

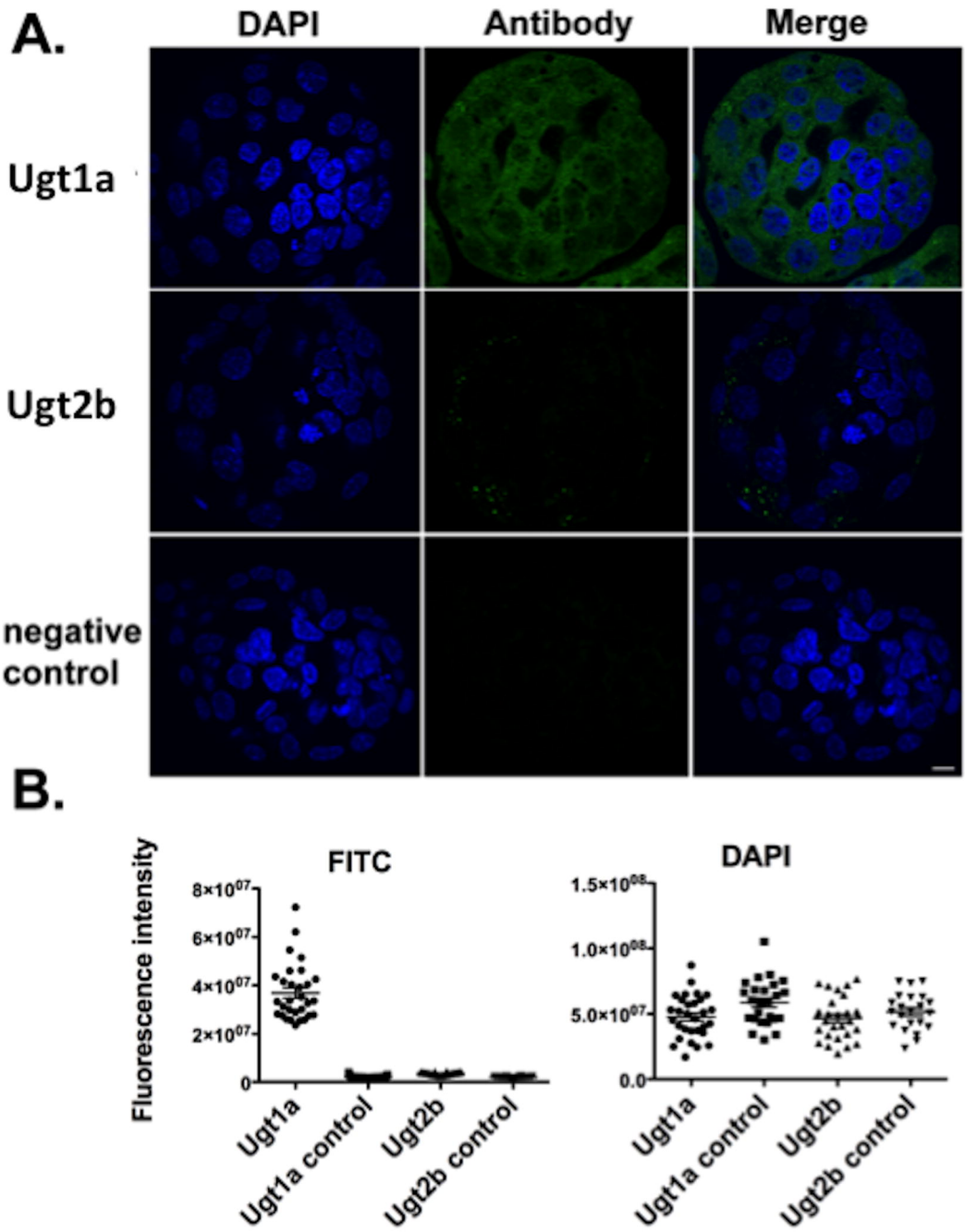
A.
DAPI
Antibody
Merge
Ugt1a
Ugt2b
negative control
B.
FITC
DAPI
Fluorescence intensity
8×10^07
6×10^07
4×10^07
2×10^07
0
1.5×10^08
1.0×10^08
5.0×10^07
0.0
Ugt1a
Ugt1a control
Ugt2b
Ugt2b control

Figure 1

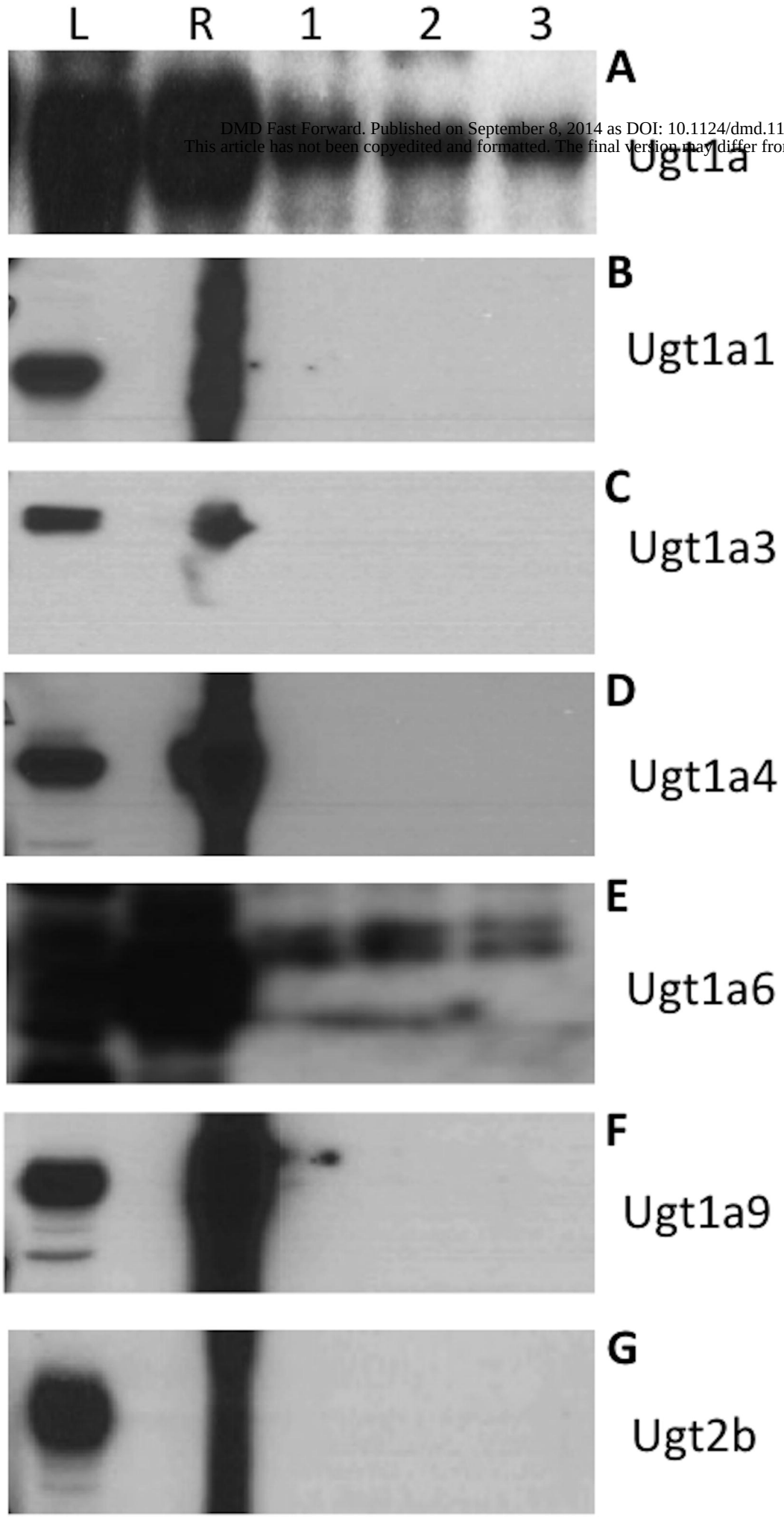


Figure 2

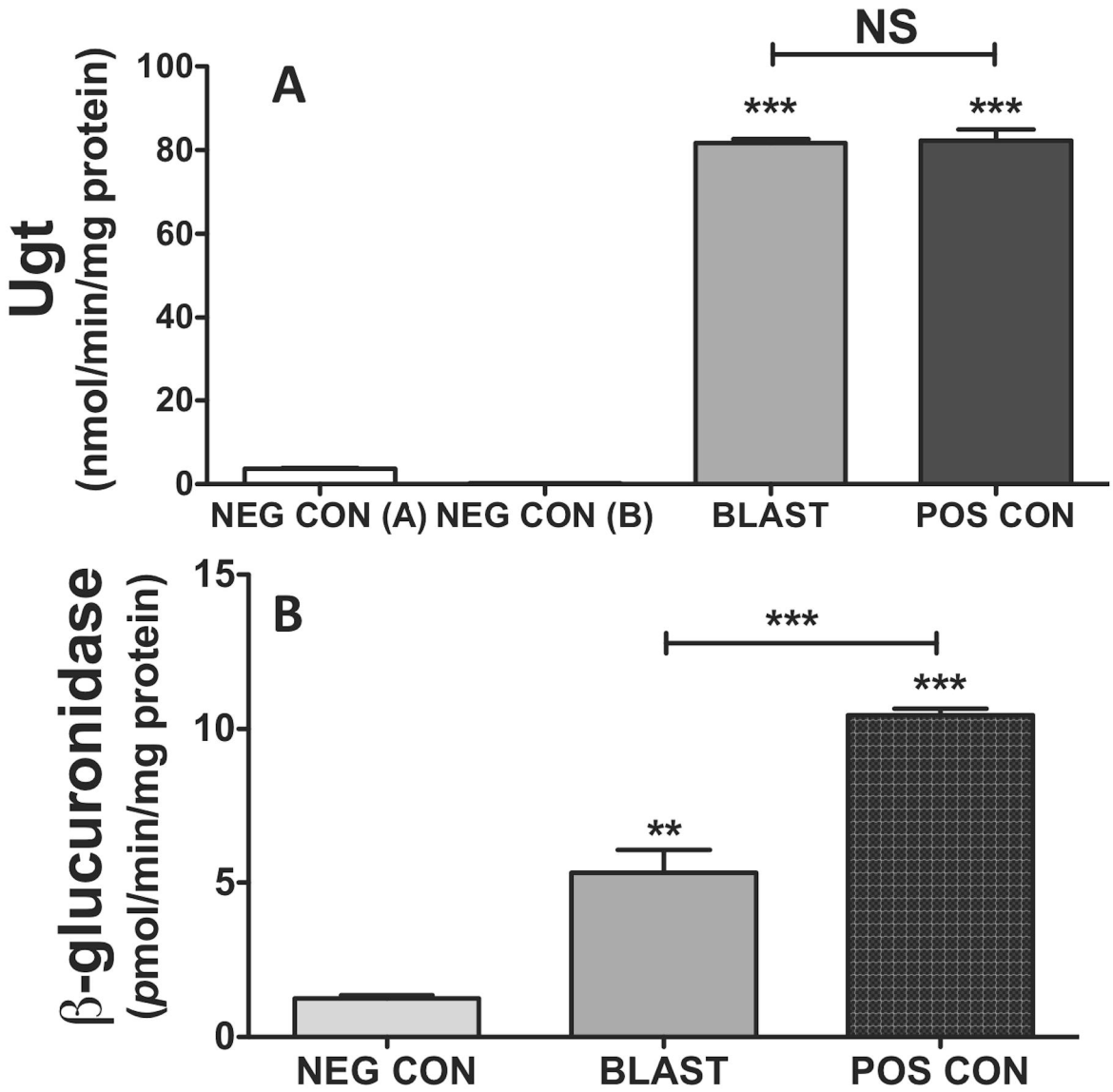
A
Ugt
(nmol/min/mg protein)
100
80
60
40
20
0
NS
***
***
NEG CON (A)
NEG CON (B)
BLAST
POS CON
B
β-glucuronidase
(pmol/min/mg protein)
15
10
5
0
***
***
**
NEG CON
BLAST
POS CON

Figure 3