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## Dolutegravir and rat whole embryo culture

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Dear Editor:

We were interested to read the recent paper by Posobiec *et al.* in *Birth Defects Research*, that reports a lack of teratogenicity using the anti-HIV drug, dolutegravir (DTG) in rat whole embryo culture. This is clinically important, as an elevated rate of neural tube defects (NTDs) was observed among women in Botswana taking a DTG-based regimen from conception [1, 2]. While further surveillance of DTG usage in pregnancy is vital and ongoing, there is also a pressing need for experimental studies to determine whether the association of NTDs with DTG exposure in early pregnancy is a cause-and-effect relationship.

We recently published a study of DTG exposure during pregnancy in mice on a folate sufficient diet [3]. Exposure to DTG at a level (1x-DTG) that delivers blood concentrations close to those seen in humans led to fetal NTDs at a similar frequency (5 cases among 1174 fetuses: 0.43%) as in humans. Other defects, particularly microphthalmia, severe edema, and vascular/bleeding defects, also showed elevated frequencies in the 1x-DTG dosing group. A five times higher dosing level (5x-DTG) did not produce more fetal abnormalities than vehicle-treated controls, possibly due to higher folate concentrations in the 5x-DTG fetuses.

Posobiec *et al.* conclude that DTG lacks teratogenicity, but this deserves closer scrutiny in view of their study design and sample numbers. First, we note that 16 embryos were cultured in each of two DTG treatment groups (5.3 and 9.3 µg/mL), compared with a vehicle

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only group, and valproic acid-treated positive controls. Based on our in vivo findings in mice, a sample size of 16 would be expected to yield 0.07 NTDs: that is, no NTDs would most likely be observed, even if the NTD rate was the same as in our in vivo study. Hence, a similar low NTD rate cannot be excluded based on the authors' chosen sample size.

Second, the authors showed that a relatively small amount (5-6% maximum) of DTG in the culture medium actually reached the embryo itself. The yolk sac, which surrounds the neurulation stage rodent embryo, appears efficient at preventing DTG entry. An extension of the study could have included DTG injection into the amniotic cavity, to bypass the yolk sac barrier [4].

Third, Posobiec *et al.* have assumed that DTG itself, and not a metabolite, is the factor to be tested for teratogenicity. However, some agents (e.g. cyclophosphamide [5]) need to be transformed by maternal metabolism, before gaining teratogenic action. Such metabolism would be expected in vivo but would not have been present in the rat embryo cultures. The rats which donated serum for use as culture medium could have been dosed with DTG to provide serum containing putative DTG metabolites, as a test of this idea.

In view of these study design concerns, we would interpret the findings of Posobiec *et al.* with caution. A teratogenic effect of DTG remains a possible explanation for the higher prevalence of NTD observed in women exposed from conception.

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