

**Optimizing Dosing Initiation in Rabbit Embryo-Fetal Development
Toxicity Studies: Avoiding the Pre-implantation Window**

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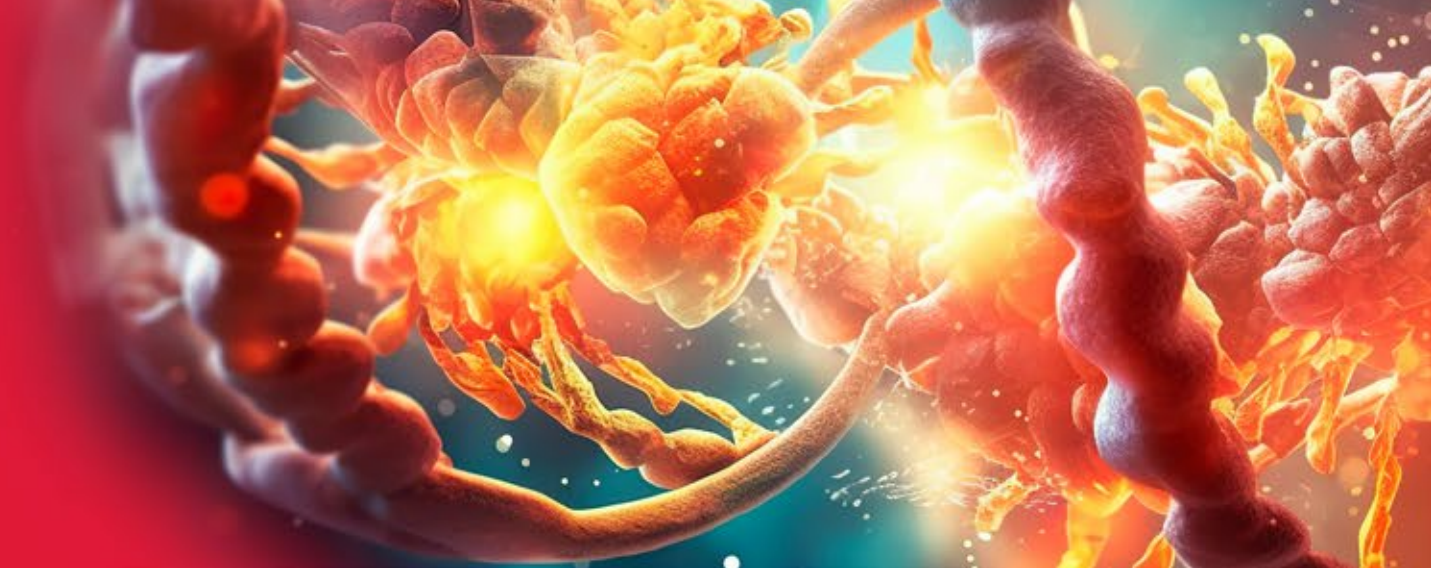


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BACKGROUND & OBJECTIVE

According to ICH S5(R3), embryo-fetal development (EFD) toxicity studies are required in both rodents and non-rodent models (typically rabbits). The assessment stage, covers the period from implantation to closure of the hard palate, and the recommended dosing period for rabbits is specifically suggested as GD (Gestation Day) 6/7 to GD 19. However, our facility observed two instances of significantly reduced pregnancy rates in rabbit EFD studies that employed the GD 6-GD 19 dosing regimen. Therefore, this integrated approach aims to provide a critical analysis and discussion on the time of dosing initiation in rabbit EFD studies.

METHODS

A retrospective analysis was performed on 66 GLP-compliant EFD studies in New Zealand White rabbits from our facility (2020-2025). Data from control groups, including the dosing initiation day (GD 6 or GD 7) and the corresponding pregnancy rates, were compiled and analyzed. For the two rabbit EFD studies, initiating dosing on GD 6 demonstrated reduced pregnancy rates. A detailed analysis of the data and potential underlying causes was performed in combination with rabbit EFD studies of 54 FDA-approved new drugs between 2023 and 2024, and also included data from rabbits in our facility.

RESULTS

1. Historical Data Analysis Reveals a Generally Stable Test System

A summary of 66 GLP-compliant EFD studies in rabbits revealed that 62 out of 66 studies initiated dosing on GD 6, with control group pregnancy rates (24 animals/group/study) ranging from 61.5% to 100% (Mean \pm SD: 88.8% \pm 10.2%). The remaining 4 out of 66 studies initiated dosing on GD 7, and the control group animals exhibited pregnancy rates ranging from 79.2% to 95.8% (Mean \pm SD: 89.6% \pm 7.2%). The overall pregnancy rates demonstrated no marked differences compared to those observed in studies initiating dosing on GD 6, and the natural conception rate in rabbits remains relatively stable.

2. Case Studies Illustrating the Risk Associated with GD6 Dosing Initiation

Despite the overall stability of the test system, two projects exhibited severe pregnancy loss linked to GD 6 dosing initiation. For a GnRH receptor antagonist study, GD 6-GD 19 dosing resulted in 0% pregnancy in the high-dose group in the pEFD study. However, in the subsequent GLP study, initiating dosing on GD 7 achieved viable litters in all groups ($n \geq 16$). Similarly, for a non-hormonal compound, the pEFD study (dosing GD 6-GD 19) showed reduced pregnancy rate (50%) at the high dose without maternal toxicity, which was restored to 91.7%–100% in the GLP study at higher doses by shifting the dosing initiation to GD 7. These cases underscore that for compounds with potential or uncharacterized effects on implantation, initiating dosing on GD 7 is a critical strategy to avoid pre-implantation loss and ensure reliable teratogenicity assessment.

3. Industry Trends and Biological Evidence Support GD7 Initiation

A review of new drugs approved by the FDA in 2023-2024 identified 54 compounds for which rabbit EFD studies were conducted. Among these, dosing commenced on GD 6 in 19/54 (35.2%) studies, while 35/54 (64.8%) initiated dosing on GD 7. This distribution indicates that, while operating within regulatory guidelines, nearly two-thirds of recent development programs prioritize the post-implantation period for teratogenicity assessment.

RESULTS

To investigate this, an internal study in rabbits ($n=6$) found that uteri on GD 6 were indistinguishable from non-pregnant animals, with no implantation sites or blastocysts observed on the endometrial surface of the uterine lumen; however, by GD 7, implantation sites become macroscopically identifiable as distinct swellings on the uterine wall. Thus, initiating administration on GD 6 carries a substantial risk of compromising pregnancy outcome.

CONCLUSION

While ICH S5(R3) provides flexibility for initiating dosing in rabbit EFD studies on GD 6 or GD 7, our integrated analysis demonstrates that GD 6 administration coincides with a critical and vulnerable pre-implantation window. This poses a substantial and avoidable risk of compromising pregnancy rates, particularly for compounds with potential effects on implantation, thereby jeopardizing the primary objective of the EFD study—the assessment of teratogenicity. Therefore, we strongly recommend initiating dosing in rabbit EFD studies on GD 7 as a standard practice.

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