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The relevance of experimental reproductive studies in safety assessment

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Abstract

Traditional methods for assessing reproductive toxicity in preclinical species are of general proven worth and human relevance. Current ICHS5(R2) reproductive studies for pharmaceuticals have a minimalist design compared to other sectors and could benefit from re-consideration of longer dosing periods especially for drugs intended for chronic administration, single gender testing and other more minor modifications. There is poor understanding of the effects of most pharmaceutics on human reproductive health but case examples exist where human monitoring, especially semen studies, have facilitated approval and patient use. Better methods are needed to assess long term effects, particularly on ovarian reserve and female reproductive health. Novel methods of sperm assessment that are potentially translatable across species require greater consideration. Based on emerging understanding of genomic alterations that impact human health and disease, future reproductive test methods may eventually require modification to accommodate new tests of germline integrity.

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Reproductive toxicity, ICH S5(R2), OECD 443, Male fertility, Female fertility.

1. Introduction and scope

For this review "Reproductive studies" is taken to mean those studies that investigate the ability of parental animals to mate, conceive and produce viable offspring, usually through rodent mating studies known colloquially as "fertility" studies [1]. The focus is almost entirely on pharmaceutical assessment, predominantly the ICHS5(R2) [2] study entitled "Fertility and Early Embryonic Development study" (FEED), with translational examples mostly from pharmaceuticals. Assessment of the effects of a test chemical on the reproductive capacity of in utero exposed first generation offspring or second generation is outside this review's scope, although key design elements of the ICHS5 pre and post natal development (PPND) study and relevant OECD guidelines are mentioned where appropriate.

Since adoption of ICHS5, there has not been a high profile human tragedy where preclinical fertility testing was proven deficient. Perhaps because of this, the Concept Paper for revising ICHS5(R2) [3] does not propose a major overhaul of FEED designs but does mention options such as incorporation of rat male functional fertility evaluation into repeat dose toxicity studies plus single gender female fertility testing.

This opinion paper reviews some limitations of current fertility study designs, provides support for ICHS5(R2) revision, gives examples where human reproductive toxicity data has informed risk assessment and touches on how scientific advancements might eventually influence reproductive toxicity assessment.

2. A critical look at critical fertility study design elements

2.1. Pharmaceutical reproductive studies have so many opt outs

ICHS5(R2) FEED studies require remarkably few reproductive endpoints. Some of the legacy "Segment 1" designs that preceded ICHS5 included evaluation of F1, but these evaluations became subsumed into the ICHS5 pre- and postnatal (PPND) study design [1]. The OECD extended 1 generation study [4] encompasses the same parental preconception, gestational and lactational exposure that is covered by the ICHS5(R2) FEED and PPND designs. Table 1 illustrates that the OECD 443 mandates several reproductive endpoints of proven sensitivity that are entirely optional in ICH study designs. The somewhat surprising outcome is that there may be less preclinical reproductive data for a pharmaceutical than for an agrochemical or a high tonnage chemical.

To improve the robustness of the risk assessment, various adaptations to pharmaceutical testing are proposed in Table 2, most of which are discussed later.

2.2. Premating dose duration- too short for comfort?

The ICHS5 guideline [2] permitted the reduction in the pre-mating dose duration in the male from the

Table 1 Examples of Reproductive endpoints[a] in adult rodents in different study designs.

	Parental animals		F1 offspring	
	ICHS5(R2) FEED	OECD 443	ICHS5(R2) PPND	OECD 443
Testes & epididymal weights	Optional	**Yes**	No[b]	**Yes**
Testes & epididymal histopathology[c]	Optional	**Yes**	No	**Yes**
Prostate & Seminal vesicle weight	No	**Yes**	No	**Yes**
Prostate & Seminal vesicle histopathology	No	**Yes**	No	**Yes**
Sperm assessment	Optional	**Yes**[d]	No	**Yes**
Oestrous cycles in unpaired animals	Optional	**Yes**	No	**Yes**
Ovarian weight	Optional	**Yes**	No	**Yes**
Ovarian histopathology	Optional	**Yes**	No	**Yes**
Primordial follicle count	No	No	No	**Yes**
Mating data[e]	**Yes**	**Yes**	**Yes**	Triggered[f]

[a] Useful References supporting OECD 443 design [80–84].
[b] No – means endpoint was not explicitly mentioned either as a required or optional endpoint.
[c] At least in high dose and control animals.
[d] Unless existing data show sperm parameters unaffected in a 90-day study; useful reference: Chapin et al., 1998 [85].
[e] Mating data includes precoital interval, insemination rate, pregnancy rate, implant number and viability assessed either prenatally or following natural birth.
[f] Retrospective analyses [80,81] have shown that assessment of an F2 generation rarely provides significant new information, hence assessment of F2 is not required in baseline data set for OECD 443. Scientific guidance on triggers for F2 generation are available [83,84].

traditional 8–10 weeks (covering the entire duration of spermatogenesis, a period broadly similar in rats and humans) to the modern 2–4 week premating dose duration protocols. This fundamental shift was justified by a literature review [5] and by collaboration studies from Japan [6,7] of male reproductive toxicants which revealed that gonadal weights and histopathology detected the majority of male (or female [8]) reproductive toxicants after only 2–4 weeks of administration.

In consequence, the current ICHS5(R2) fertility study guidance allows a 2 week premating protocol for both genders "Provided no effects have been found in repeated dose toxicity studies of at least 2 weeks duration that preclude this". The 2 week protocol covers approximately 3 rodent ovulation cycles (which has seldom been challenged as too short for assessment of female mediated effects), however, few pharmaceutical testing laboratories routinely use a 2 week premating protocol for males – it is intuitively perceived as a less robust design than a 4 week premating duration for novel candidate drugs (CDs).

2.3. Challenging the basis for short -premating dose duration

In the Japanese collaborations [6,7], compounds affecting rodent spermatogenesis predominated, which by their mode of action (MOA) are likely to cause overt testicular histopathological changes in short duration studies. However, CDs can have profound effects on male reproductive function without histopathology effects [9,10] and for this universe of molecules, with diverse MOA, there is insufficient precedent to say whether 2 week pre-mating dose duration is generally sufficient to unequivocally detect a hazard and provide a robust NOAEL for chronic administration to man [9].

Because of the orderly nature of spermatogenesis in the rat, it is easier to spot subtle test article related histopathological signals in the rat testes than in the dog or monkey [11]. Female rodents typically have a 4 day cycle whereas monkeys or bitches, ovulate approximately once a month or twice a year respectively, so again, reproductive tract signals from a non-rodent repeat dose toxicity study of 2–4 weeks duration may not be as informative as rat [12]. Coupled to this, "First time in Man" repeat dose toxicity studies often have group sizes of 10 in rodents versus only 3 in the non-rodent which exacerbates difficulties in non-rodent signal detection. Another impediment to signal detection in non-rodents is the use (see Table 2) use of sexually immature animals – either through ignorance, costs or logistical expediency.

A more recent analysis [13] of "real-life" candidate drugs (CDs) comparing the target organ profiles of First Time in Man studies (typically 2–4 week duration studies) versus subchronic/chronic studies reveals that even within a relatively small dataset of 39 CDs, there were 10 molecules with changes in the male reproductive organs and 4 instances where histopathological change in testes or epididymides were only detected in the longer duration studies in either rodent or non-rodent. The overall incidence of changes in the female reproductive tract (including mammary) was only 5, but the majority were newly revealed in the longer duration studies.