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Timing is everything for sperm assessment in fertility studies

Dirk Mariën[a,*], Graham P. Bailey[a], Gary Eichenbaum[b], Sandra De Jonghe[a]

[a] Preclinical Development & Safety, Janssen Research & Development, Beerse, Belgium
[b] Preclinical Science & Safety, Office of the Chief Medical Officer, Johnson & Johnson, New Brunswick, NJ, USA

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ABSTRACT

The fertility study design recommended in the ICH S5(R2) Harmonised Guideline for Detection of Toxicity to Reproduction for Medicinal Products emphasizes the importance of histopathological endpoints next to a pairing assessment in evaluating male fertility. However, in a male rat fertility study with JNJ-26489112, a CNS-active agent, while there were no effects on histological endpoints, mating performance or pregnancy outcomes, sperm assessment was included. The high dose males presented with reversible decreases in epididymal, but not testicular, sperm concentration and motility and an increase in abnormal sperm morphology. In view of the differences in fertility between rats and humans, these types of sperm effects in rats suggest the potential for an impact on human male fertility that would be undetected if not for the sperm assessment. Therefore, the current example suggests that including semenology as a standard endpoint in nonclinical fertility studies may be warranted.

1. Introduction

The objective of the nonclinical fertility study is to evaluate potential effects of pharmaceuticals on general reproductive performance and fertility in male and female animal models. The assessment for candidate drugs is generally conducted in rats and is guided by the ICH Harmonised Tripartite Guideline on the Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility S5 (R2) [1,2]. The guidance does not present mandatory rules, but does provide basic rules and guidance, including several scenarios and possible alternatives that should be considered depending on the existing data on toxicity, pharmacodynamics, kinetics and similarity to other compounds in structure/activity [1–4]. The guidance specifically addresses evaluation of toxicity to male fertility, recommending study designs that "*will permit detection of functional effects* (*e.g. on libido, epididymal sperm maturation*) *that may not be detected by histological examinations of the male reproductive organs*".

While a combined male and female fertility study, with both sexes treated in a single study, may be sufficient in many cases, separate and more comprehensive male and female fertility studies investigating possible effects of treating either sex alone may be necessary to distinguish between male- or female-mediated effects. The decision on whether to conduct a combined or separate study is generally driven by existing knowledge from previously conducted 28-day or longer repeated dose toxicity studies, such as effects on reproductive organ weights, histopathological changes or mode of action suggesting a potential reproductive toxicity issue.

In the most commonly used, combined fertility study design female rats are dosed for 2 weeks prior to pairing, during pairing and at least through implantation (generally Day 6–7 of presumed pregnancy). Females are necropsied for examination of their uterine content at the mid-point of pregnancy. Male rats are dosed for 2–4 weeks prior to pairing, during pairing and are generally continuously dosed until the females are sacrificed and the fertility rate can be confirmed. This allows, depending on the pregnancy outcome, for further investigations on the treated males such as a second pairing assessment (e.g. with untreated females) or sperm examination for confirmation or exclusion of male-mediated effects. In the latter case, it should be noted that the males could eventually be treated for up to 8 weeks, and possible deficiencies noted at sperm examination at this time point may not necessarily reflect the situation at the time of pairing (i.e. after only 2–4 weeks of dosing). If effects are noted on the pregnancy outcome, directly correlating these with any changes observed at sperm examination is precluded because of the delayed sperm evaluation at 7 or 8 weeks that is inherent to the study design.

In this publication, a case example is presented where such an issue of timing for male fertility assessment arose. JNJ-26489112, a sulfamide derivative, is a central nervous system (CNS) active compound that has in vitro activity at multiple CNS targets including NMDA, kainate, GABA, Na Type II, KCNQ and N-type calcium chan-

* Corresponding author.
E-mail address: dmarien5@its.jnj.com (D. Mariën).

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nels. In addition, JNJ-26489112 demonstrates modest activity at the 5-HT2c, dopamine transporter and dopamine and serotonin uptake sites. The compound showed efficacy in multiple preclinical models for depression, epilepsy, neuropathic pain, inflammatory pain, migraine and anxiety [5].

Based on the approach provided by the ICH guidance and the evidence of (histo)pathological changes from previously conducted 1- and 3-month repeated dose toxicity studies on the female genital tract, a separate female fertility study was performed to investigate the impact of JNJ-26489112 on reproductive functions. The design and results of this study will however not be discussed as this is beyond the scope of this paper. With regard to male fertility there were no indications from the repeated-dose toxicity studies in either rodents or non-rodents (no effects on testis, epididymides or accessory sex gland weight or histopathology and no indication for any effect on testosterone metabolism) or the mode of action of JNJ-26489112 for a potential effect on male fertility. Therefore a 4-week pre-pairing dosing design was adopted according to the ICH S5 guideline: "*Provided no effects have been found in previously conducted repeated dose toxicity studies, a pre-mating treatment interval of 4 weeks for males can be used.*" even though the addendum (part II) of the guideline on toxicity to male fertility mentions 2 weeks to also be acceptable as it was validated to be as effective as a 4-week premating treatment period. This reasoning implies that in absence of any (histo)pathological findings or anticipated effect on the male reproductive organs, any functional defect would be picked up by dosing for 2–4 weeks prior to mating.

The study design and results from the initial study and the subsequent time-course investigations to elucidate the timing of onset and the reversibility of the recorded fertility effects are described in this paper.

2. Materials and methods

2.1. Repeated dose toxicity studies

Before the start of the fertility studies, repeat dose toxicity studies with durations of 1, 3 and 6 months were conducted to assess the potential toxicity of compound JNJ-26489112 in support of its clinical development. These studies were designed in accordance with OECD guidelines for the testing of chemicals (Nos. 407 and 408).

For this purpose, Sprague Dawley rats [Crl:CD(SD)] received once daily oral (gavage) administrations at the dose levels indicated in Table 1.

A fourth group of animals received the vehicle alone at the same regimen and served as vehicle control group. Assessments were made for mortality, clinical observations, body weight, food consumption, ophthalmoscopy, hematology, coagulation, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology. In addition in the 1-month study, reversibility of any observed effect was assessed after a 1-month recovery period. Satellite groups of rats were included in all studies for toxicokinetic evaluation.

2.2. Male fertility studies

The fertility studies were conducted at Janssen Research & Development (Beerse, Belgium) in compliance with the current "Good Laboratory Practice" principles of the OECD GLP guidelines. Janssen R&D's vivarium facilities are AAALAC approved and all animals were treated humanely and cared for in accordance with the European and Belgian guidelines, and with the principles of euthanasia as stated in the Report of the American Veterinary Medical Association Panel. Male and female Sprague Dawley rats [Crl:CD(SD)] were obtained from Charles River (Germany) and housed in an environmentally controlled SPF rodent facility with a 12 h light/dark cycle. The males were housed in polysulphon rat cages with a wire-mesh lid, a floor area of approximately 3000 cm^2, provided with bedding material (Corn Cob size 12, Eurocob, France) and suspended in wheeled racks. The males were group-housed (3–5 rats/cage) during the pre- and post-pairing periods, and individually with the untreated female (1:1) in the females cage during the pairing period. The females were housed individually (except during the pairing period) in polysulphon cages with a wire-mesh lid, a floor area of approximately 940 cm^2, provided with bedding material and suspended in wheeled racks. During the pairing period, a wire mesh floor was provided to enable counting of copulation plugs. The rats were given free and continuous access to water and feed (R/M-H pelleted maintenance diet; Ssniff, Soest, Germany).

The formulations were aqueous suspensions with 0.5% (w/v) Methocel (hydroxypropyl methylcellulose) in demineralised water and were administered by oral gavage at a dose volume of 10 ml per kg body weight.

2.2.1. Initial male fertility study

Following an acclimatization period of approximately 1 week, the male rats, aged approximately 8–9 weeks at arrival, were randomized into 4 groups based on initial body weight. Groups of 22 males each received once daily doses of 30, 100 or 300 mg/kg/day by oral gavage for 4 weeks prior to pairing, throughout the pairing period and up to termination (i.e. dosing period of approximately 8 weeks in total). A fourth group of animals received the vehicle alone following the same regimen and served as vehicle control. The males were monitored daily for clinical signs of toxicity throughout the study. Body weight was recorded weekly throughout the study and food consumption weekly during the pre-pairing period (Fig. 1).

Untreated female rats, aged approximately 8–9 weeks, were assigned randomly to the treated males (1:1) in the late afternoon after completion of the 4 week male treatment period for pairing assessment. Daily vaginal smears (vaginal lavage using tap water and disposable pipettes; stained with New Methylene Blue N 0.5% [Aldrich]) were taken the following morning and each subsequent morning and evaluated for evidence of mating by the presence of sperm, and to determine the stage of oestrous. The day of mat-

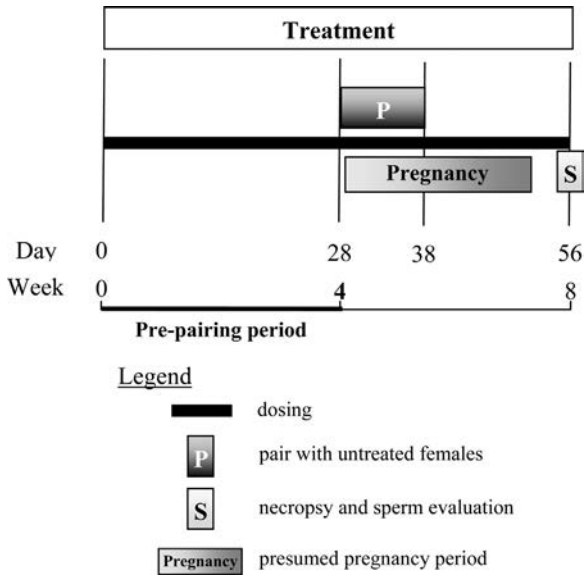

Fig. 1. Initial fertility study: timing.