Letter - Consistency of gonadotrophin products - K Gordon et al.

We fully concur with Keith Gordon and colleagues (this issue, Gordon et al., 2005) concerning the precision of in-vivo bioassays defined by e.g. the European Pharmacopoeia. Presumably, the in-vivo bioassay best defines the FSH potency, since both the biological action at the target tissue and the rate of biological clearance are taken into account. In fact, bioassays are still the only way to measure the integrated physiological response of an isoform mixture of gonadotrophins on its end organ. For example, Wide and Hobson (1986), among others, have shown that the more acidic forms (higher content of sialic acid) of human FSH exhibit longer plasma half-lives and higher in-vivo bioactivity than the less acidic isoforms (Wide, 1986; Wide and Hobson, 1986; Galway et al., 1990; Bishop et al., 1995). Bassett and Driebergen (2005) appear to agree that this is the case and point out that the distribution of isoforms may affect the specific biological activity (Basset and Driebergen, 2005). In this context, it is important to note that the variation in the isoform distribution appears to fluctuate extensively (Figure 2, Bassett and Driebergen, 2005) in Gonal-F®. In fact, it would appear that the variation of individual isoform bands can vary as much as ±30% around the mean value (either due to lack of precision of the method or due to a real variation in the isoform profile). Such large variations in individual isoforms would undoubtedly affect the biological potency as measured by bioassay, potentially without affecting the protein content.

Furthermore, as mentioned by Gordon et al., (2005), it is indeed difficult to understand how this new fill-off procedure (filled-by-mass; FbM) would result in an improved clinical outcome. As mentioned above, even though a gonadotrophin product has a constant protein content, there can still be a large variation in the biological activity between drug product batches. The protein content may of course be different between batches when filling by bioassay (FbIU), since the bioassay measures the integrated physiological response of an isoform mixture of gonadotrophins on its end organ (if there is a large isoform variation between gonadotrophin batches). However, the protein content in each vial within a FbIU batch will not vary more than a FbM batch, since the variation between vials within a batch is dependent on the precision of the actual filling procedure and not the protein content or bioassay value variation. Therefore, it is reasonable to assume that the variation in ovarian response within one batch should be similar between FbIU and FbM, since the protein content variation between vials within a batch is presumably the same regardless of whether the vials are filled by mass or bioassay. This does not appear to be the case in Figure 2 in the paper from Hugues et al. (2003) (e.g. FbIU batches BFDA98554 and BFDA98536). Instead, the variation per batch would appear to be primarily associated with the variation in response between patients. It is also interesting to note that there is a difference in average oocytes retrieved between batches with far less than the variation in response between patients per batch. This indicates that the numerical difference seen in variation of average number of oocytes retrieved between FbM and FbIU batches was likely obtained by chance, as suggested by Gordon et al. (2005).

Finally, if the findings of Wolfenson et al. (2005) translate into actual differences in biological potency between Gonal-F® FbM and FbIU (Wolfenson et al., 2005), the safety and efficacy of Gonal-F® FbM should be confirmed in controlled clinical trials.

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References


Hugues J-N, Barlow D, Rosenwaks Z et al. 2003 Improvement in consistency of response to ovarian stimulation with recombinant human follicle stimulating hormone resulting from a new method
Consistency of gonadotrophin products

To the Editor

It is with pleasure that I take the opportunity to reply to the letter from Dr Gordon and colleagues (this issue, Gordon et al., 2005). The objective of our article (Bassett and Driebergen, 2005) was to demonstrate that the development and use of ‘fill by mass’ (FbM), as a manufacturing process for follitropin alfa, successfully delivers the most consistent recombinant human FSH (r-hFSH) in terms of protein content. The results convincingly showed that follitropin alfa FbM had a superior batch-to-batch consistency (1.6%) than follitropin beta (12%).

Contrary to the statement from Gordon et al. (2005), it is incorrect to state that the precision of the bioassay, as used by Serono, was in the range of 20% or that the large imprecision was the reason that Health Authorities requested Serono to develop a more precise fill process. The lack of precision for the bioassay was indeed discussed by Driebergen and Baer (2003) who stated that the coefficient of variation (CV) for a single determination is 10–20%. Furthermore they indicated that multiple tests are required to achieve lower variability. In fact Mulders et al. (1997) stated that 6–10 standard experiments and 360 rats are needed to achieve a dose accuracy of less than 10%. This fact is graphically demonstrated in Figure 1 of the same paper, where the CV is 10% for eight independent bioassays. Therefore it would be interesting to reflect on the number of replicate bioassays and sacrificed animals that are needed to achieve a precision (CV) of less than 5% (Gordon et al., 2005). In fact it is difficult to find any clear statement in the references quoted by Gordon et al. (2005) that the follitropin beta bioassay has achieved a precision of less than 5%. The bioassay variability is intrinsic to the analytical procedure and both Driebergen and Baer (2003) and Mulders et al. (1997) state this clearly.

During the approval process of follitropin alfa, in Europe and the USA, the regulators surmised that the potency of such a well-characterized biotechnology-derived FSH could be assessed using a physicochemical method rather than the imprecise bioassay. The regulatory recommendation was not as a consequence of an existing ‘large batch-to-batch variation’ as incorrectly stated by Gordon et al. (2005), but due to an understanding that the bioassay was inherently imprecise; and a pure, consistent, and well characterized protein may be suitable for such a physicochemical approach to vial filling. This was not a surprise as most biotechnology-derived therapeutic proteins use physicochemical analytical methods to assess the drug content, and there is a general desire to reduce or eliminate the use of animals for drug testing (Castle, 1998; Artiges, 1999). This physicochemical approach is further supported by the ICH guideline Q6B (ICH Topic Q6B, 1999) where it is clearly stated that ‘Quantity, usually measured as protein content, is critical for a biotechnological product … using an appropriate assay, usually physicochemical in nature … it may be appropriate to use measurement of quantity rather than the measurement of biological activity in manufacturing processes, such as filling’. However, because of the complexity of glycoproteins, such as FSH, it had until recently been believed that physicochemical alternatives were problematical (Bristow and Charton, 2002) or that they may not be able to meet pharmacopeial requirements (Wijn et al., 2002).

The development and introduction of follitropin alfa FbM was based on the consistent r-hFSH isofrom distribution of the drug substance, and the demonstration that the protein content as assessed by a very precise size-exclusion assay (CV 1–2%; Driebergen and Baer, 2003) gave a consistent relationship to the biopotency. This FbM approach translated into a consistent drug product where the variability for follitropin alfa was assessed as 1.6% over 30 different drug batches (Bassett and Driebergen, 2005). The manufacturers of follitropin beta (Organon) themselves have investigated the use of physicochemical techniques, capillary zone electrophoresis (Wijn et al., 2002), and isoelectric focusing technique (Mulders et al., 1999), as an alternative to the in-vivo bioassay; however, unfortunately to date there seems to be no information on its performance.

To summarize, the consistency of the FbM process (1.6%) is substantially better than the documented consistency of follitropin beta filled by bioassay (10%) (Mulders et al., 1997). It is also noted by Gordon et al. (2005) that the original article (Bassett and Driebergen, 2005) cited several clinical studies undertaken to assess the clinical implications of follitropin alfa FbM. The conclusions from these studies support the