Commentary: standardization of nonsteroidal regulators of gonadotrophin secretion

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The concept of nonsteroidal regulators of gonadotrophin secretion originated with the identification of inhibin – a hormone produced by the gonads that selectively inhibits the secretion of pituitary follicle stimulating hormone (FSH) – the existence of which was postulated some 70 years ago (Mottram and Cramer, 1923; McCullagh, 1932). However, the purification and characterization of inhibin was a long and arduous task confounded by many factors, including the wide range of starting materials, the different bioassay systems used to define and estimate inhibin activity, the properties of the protein that rendered it resistant to standard protein purification techniques, and the absence of a generally accepted reference preparation for inhibin activity (Findlay, 1986).

In addition to being a member of a wider family of proteins with diverse biological activities in a number of systems, inhibin itself is a complex protein existing as different molecular mass forms as well as products of different genes (for example see Findlay, 1986; Mason *et al.*, 1986; Stewart *et al.*, 1986; Robertson *et al.*, 1992). Inhibin is now known to be a heterodimeric glycoprotein composed of an α subunit and a β subunit which are not covalently linked. The β subunit occurs in two different forms, termed β A and β B, giving rise to inhibin A and inhibin B on combination with the α subunit. The β subunits are also known to combine together to give rise to a molecule which is termed activin on account of its ability to stimulate FSH secretion from the pituitary. Activin can exist in at least three forms: activin A, activin AB and activin B, depending upon the β subunit composition. Robertson *et al.* (1995, 1996), using a fractionation procedure, reported the identification of a range of bioactive and immunoactive forms of inhibin in human plasma. These forms have molecular masses of 28–128 kDa, attributed to differences in glycosylation of the α subunit and differential processing of the α and β subunits. Importantly, in human female serum the majority of these forms appear to be bioactive. Robertson *et al.* (1995, 1996) conclude that fractionation of serum inhibins can be used to evaluate the specificity of assays used to study inhibin physiology and that their observations highlight the need for appropriate standards, particularly when inhibin is estimated in a clinical setting. The determination of inhibin is further complicated by the secretion and circulation of α subunit precursor proteins (Groome *et al.*, 1995; Lambert-Messerlian *et al.*, 1995). The identification of molecules that are not structurally related to inhibin, such as follistatin (Nakamura *et al.*, 1990) and α_2 -macroglobulin (Vaughn and Vale, 1993), which can act as binding proteins for activin and inhibin, may

lead to interference in both bioassays and immunoassays for inhibin and activin.

During the isolation of inhibin (for example Robertson *et al.*, 1985), research depended upon *in vitro* biological assays in which classically defined inhibin bioactivity could be detected and estimated, and this activity was displayed by the various local standards, many of bovine, pig or other non-human origin, used in this research. In 1984 the World Health Organization (WHO) Special Program of Research, Development and Research Training in Human Reproduction recognized the need for an ampouled stable reference preparation of inhibin to be made widely available and to serve as a research standard for *in vitro* bioassays of inhibin. As no standard for human inhibin was available, an extract of pig follicular fluid was provided by the National Institutes of Health (NIH) Contraceptive Development Branch, Bethesda, USA, assessed by an international collaborative study and established as the First International Standard for Inhibin, Porcine. (WHO ECBS, 1990; Gaines-Das *et al.*, 1992).

More recent developments have given rise to immunologically based assays (for example Groome and O'Brien, 1993) and novel *in vitro* assays (Smyth *et al.*, 1993), which may require highly purified inhibin reference preparations for calibration, and interest in human inhibin in various physiological situations has expanded (for example Muttukrishna *et al.*, 1995). The only source of highly purified human inhibin in sufficient quantity to serve as a standard for such assays is recombinant DNA technology subsequent to the cloning of inhibin (Forage *et al.*, 1986). Therefore, an international collaborative study including preparations of rDNA-derived human inhibin and of human follicular fluid has recently been completed (Rose and Gaines-Das, 1996) and a preparation of rDNA-derived human inhibin deemed suitable to serve as an International Standard for *in vitro* bioassays and immunoassays was established as the First International Standard for Inhibin, Human, Recombinant, with an assigned unitage of 150 000 iu per ampoule. This unitage maintains an approximate continuity with the International Standard for Porcine Inhibin (Code 86/690) in the 'original' *in vitro* bioassays.

The above International Standards for inhibin are available from the National Institute for Biological Standards and Control to support the development of assays for, and research on, the physiological and clinical importance of inhibin. The international collaborative studies that evaluated these standards have highlighted the variability that occurs among estimates made in laboratories using different standards and have also indicated that assay systems currently in use may show markedly different specificities for the various

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components of naturally occurring and recombinant DNA-derived inhibins. These findings, along with those described above, demonstrate the need for standard preparations for inhibin, including inhibin A, inhibin B, different molecular mass forms of inhibin, and for the α and β subunits of inhibin. In addition, standards for molecules such as activin and proteins that interact with inhibin, such as follistatin and α_2 -macroglobulin, and the recently identified gonadotrophin surge attenuating/inhibiting factor (GnSAF/GnSIF) (Danforth and Cheng, 1995) may be useful. However, further development of inhibin standards awaits a consensus from the scientific research community on prioritization of the potential multitude of reference reagents. Many of these materials are currently in short supply and are not available in sufficient quantities to serve as International Standards. Mechanisms for producing the required materials in large quantities should be sought. As a WHO International Laboratory for Biological Standards we welcome comments from interested parties.

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