Currently, PCOS is diagnosed by the presence of different combinations of three criteria, clinical and/or biochemical hyperandrogenism, chronic oligo-anovulation and ultrasonographic appearance of the ovaries, after exclusion of other causes. However, there is still controversy about these criteria. From the ultrasound point of view, both number of ovarian follicles and ovarian volume may be used for PCOS diagnosis, with thresholds of 12 or more follicles and > 10 ml, respectively. The aim of this study was to verify these criteria in a sample of PCOS women. A total of 124 subjects recruited in the Verona 3P Study, 90 PCOS and 34 controls, were studied. Hyperandrogenism was assessed by clinical examination and serum free testosterone levels, measured by liquid chromatography-mass spectrometry and equilibrium dialysis. Ovarian ultrasonography was carried out, whenever possible, by a transvaginal approach. The number of follicles, their diameters and the ovarian volume were measured in both ovaries, between cycle days 3 and 10 in non-amenorrheic women, or in random days in amenorrheic women. Receiver operating characteristic (ROC) curves were constructed to examine the diagnostic test performance of correctly identifying controls and patients with PCOS, and the area under the curve (AUC) was calculated. The best compromise between sensitivity and specificity for ovarian follicles was obtained with a threshold of >7 follicles (sensitivity 95.6%, specificity 97%, ROC AUC 0.996). Conversely, the threshold for the ovarian volume was >10 cm3, with lower diagnostic test performance (sensitivity 64.4%, specificity 87.5%, AUC 0.825). Our results show that the number of ovarian follicles has a better diagnostic performance than the ovarian volume for diagnosing PCOS, and that a better threshold for the number of follicles may be lower than proposed by the Rotterdam consensus.