

## ELECTRONIC SUPPLEMENTARY INFORMATION:

### *Ex vivo* detection and quantification of gold nanoparticles in human seminal and follicular fluids

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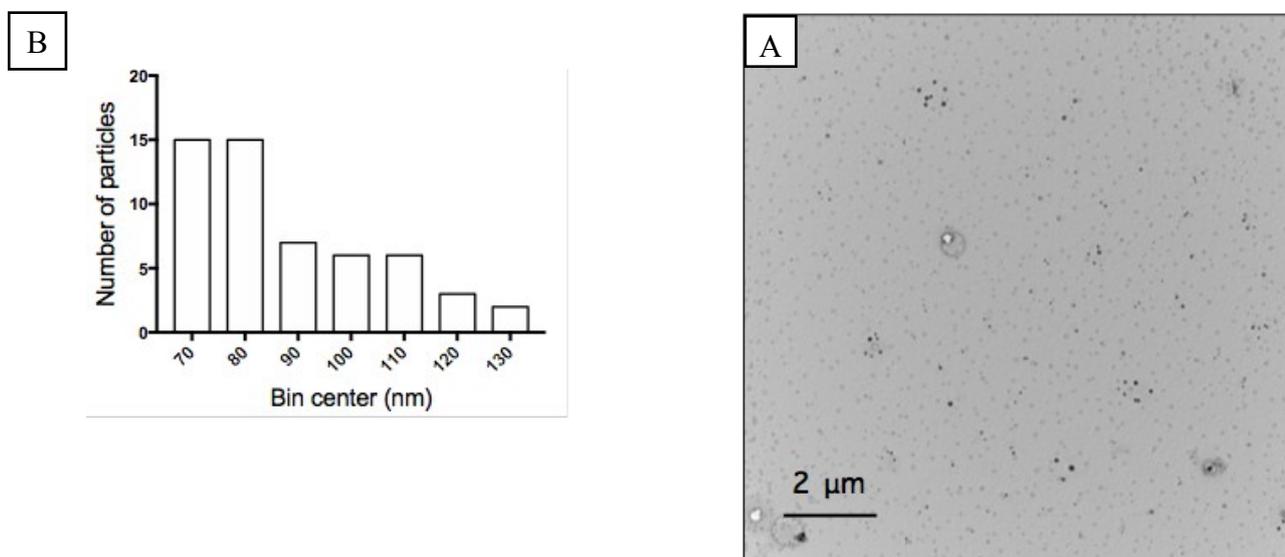
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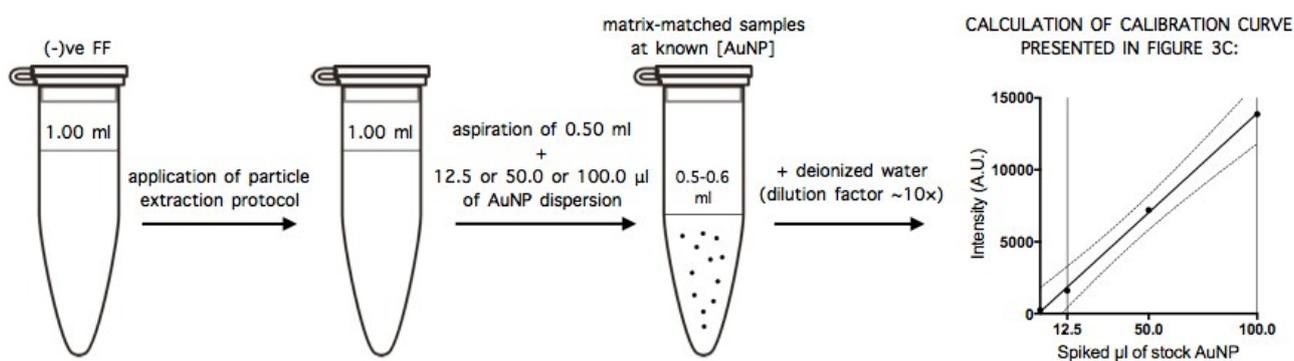
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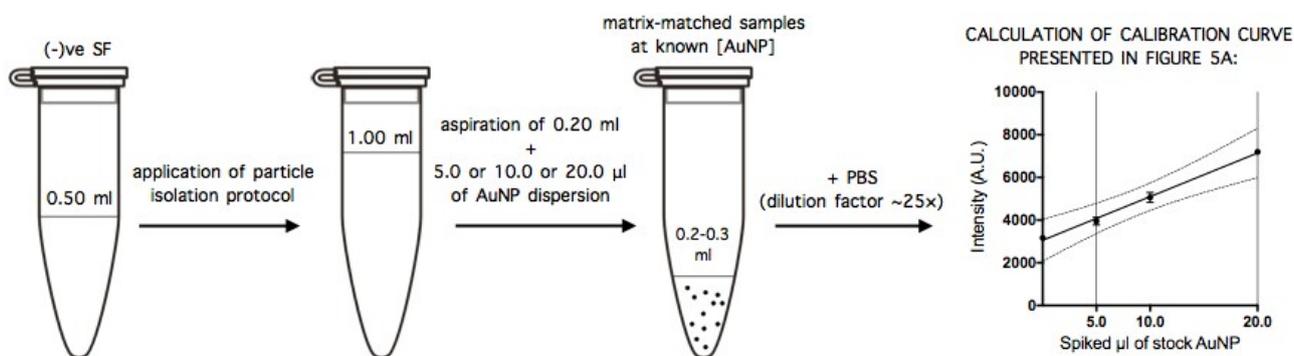
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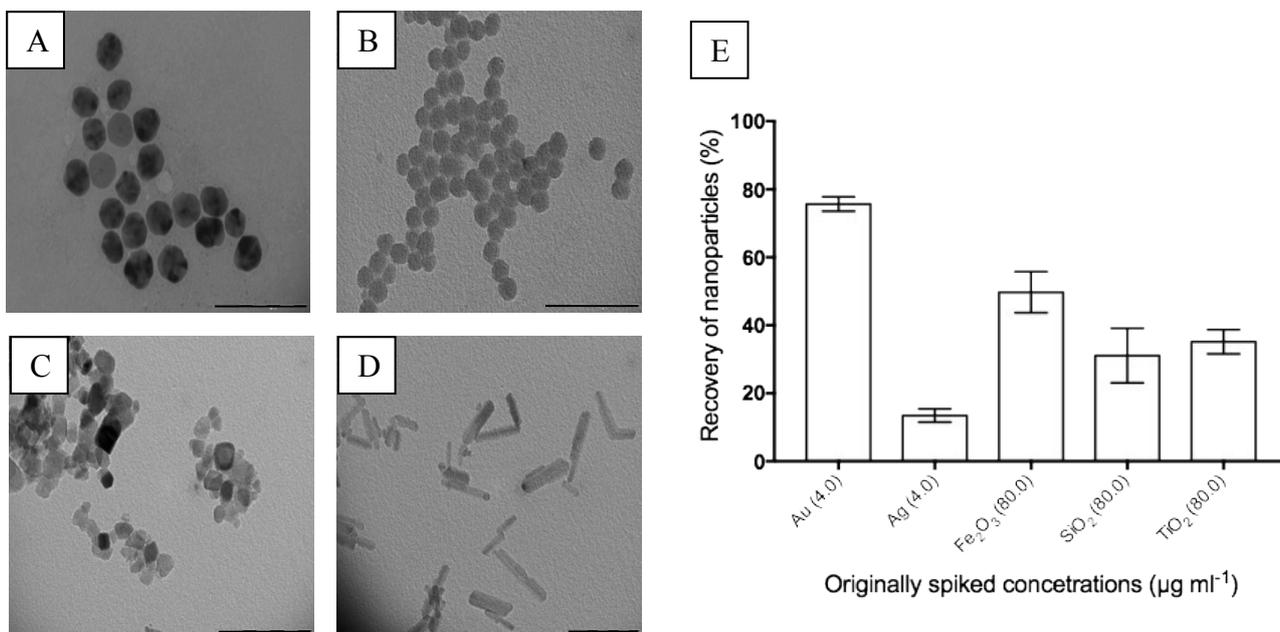
**Fig. 1** (A) Transmission electron microscopy (TEM) micrograph of gold nanoparticles (AuNP) extracted from follicular fluid (FF) as presented in the main text, but in larger format. (B) TEM micrograph of the same sample at lower magnification. (C) Frequency distribution of AuNP diameters after their extraction form FF as measured with ImageJ ( $n=55$ ).



**Fig. 2** Preparation of calibration curve for the quantification by means of inductively-coupled plasma optical emission spectroscopy (ICP-OES) of AuNP extracted from FF: initially, undiluted FF aliquots (1.00 ml) were treated according to the particle extraction protocol as presented in the main text; then, known quantities of AuNP were added in 0.50 ml of the prepared samples. These were finally diluted in deionized water before measuring their light intensity at 242.795nm by ICP-OES. Due to the initial FF aliquots volume (1.00 ml), the volume used for ICP-OES measurements (0.50 ml), and the nominal concentration of the stock AuNP dispersion ( $\sim 40 \mu\text{g ml}^{-1}$ ), the calculated calibration curve can be used for quantifying AuNP in FF in the range of  $1\text{-}8 \mu\text{g ml}^{-1}$ . All measurements were performed in triplicate and error bars represent sample standard deviation (S.D.).



**Fig. 3** Preparation of calibration curve for the quantification by means of ICP-OES of AuNP retrieved from seminal fluid (SF): initially, undiluted SF aliquots (0.50 ml) were treated according to the particle isolation protocol as presented in the main text; then, known quantities of AuNP were added in 0.20 ml of the prepared samples. These were finally diluted in PBS before measuring their light intensity at 242.795nm by ICP-OES. Due to the initial SF aliquots volume (0.50 ml), the volume used for Au quantification (0.20 ml), and the nominal concentration of the stock AuNP dispersion ( $\sim 40 \mu\text{g ml}^{-1}$ ), the calculated calibration curve can be used for quantifying AuNP in SF in the range of  $2\text{-}8 \mu\text{g ml}^{-1}$ . All measurements were performed in triplicate and error bars represent sample standard deviation (S.D.).



**Fig. 4** TEM micrographs of engineered nanoparticles tested with the proposed particle isolation method for SF: (A) Ag, (B) SiO<sub>2</sub>, (C) TiO<sub>2</sub>, and (D) Fe<sub>2</sub>O<sub>3</sub>. All scale bars are at 100nm. (E) The recovery yields for the above mentioned nanoparticles from SF were measured by ICP-OES. Their quantification was performed similarly to the process followed for AuNP, i.e., with processed SF aliquots which were then spiked with known quantities of each type of material. All measurements were performed in triplicate and error bars represent S.D.