consistent with (though admittedly give no support to) the contention that there is an upward turn on the right side of the regression. However, if Dr Bernstein wishes to deny the existence of the right arm of the regression, then she needs to provide grounds for supposing bias in the US data (because that right arm is present every year).

The explanation in the third paragraph is a possible interpretation, but for it to be plausible, evidence should be adduced that lower class men actually were away from the front getting killed, while upper class men were at home, living it up. (I am aware of contentions of this sort in regard to the US forces in Vietnam: but the high wartime sex ratios which it is trying to explain occurred in all the belligerent countries in both world wars). Moreover, the suggestion that lower class births are associated with a low sex ratio is itself contentious.

With regard to paragraph 4, I acknowledge that Boklage provides a possible explanation of the sub-binominal distribution of the combinations of the sexes in mammalian litters. However, as far as I know, there has been no confirmation of his work in other species (or ours).

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Spermatid conception

Dear Sir,

With respect to the paper by Sousa et al. (1998) in the February 1998 issue of Human Reproduction, we carefully reviewed the photographs showing 'round spermatid' injection under Hoffman optics. We do not feel that these cells can be verified to be round spermatids under Hoffman optics. There are simply no clearly definable features under Hoffman optics that can identify those round cells as spermatids.

Please see Figure 1 of this letter in which the arrows point to a Sertoli cell nucleus (seen under Hoffman optics in patients with Sertoli cell-only), which is typically misinterpreted as a round spermatid. See also Figure 2 which is a phase contrast view of a testicular sperm extraction (TESE) specimen which has mature spermatooza, Sertoli cell nuclei, and round spermatids present. Note the arrows point to the round spermatids reliably apparent by the acrosomal vesicle. Phase contrast microscopy allows a more reliable identification of cell types at TESE-intracytoplasmic sperm injection (ICSI) than Hoffman optics.

Furthermore, a careful review of all our light microscopy stained slides of maturation arrest, Sertoli cell-only, and normal spermatogenesis patients has revealed no round spermatids when there were no mature sperm, or spermatids with tails. These results were then compared with a phase contrast study of TESE specimens in all of our patients with azoospermia. Under phase contrast, one again could reliably identify round spermatids, but they were never found in the absence of mature spermatooza. This observation confirms the observations of Söderstrom and Suominen (1980) which state clearly: 'In meiotic arrest, the spermatogenic cell differentiation process seems to proceed normally up to the late pachytene or diplotene stages of meiotic prophase. However, no spermatids can be seen in the tubules ... the site of meiotic arrest was always very constant in the late meiotic prophase and did not vary even between different patients.'

This also confirms the observations of Verheyen et al. (1998) in which no round spermatids were observed in the absence of spermatozoa or mature spermatids. Therefore, we think it is time to seriously reconsider whether round spermatid nuclear injection (ROSNI) and round spermatid injection (ROSI) are solutions to non-obstructive azoospermia when no spermatozoa or mature spermatids can be identified in the TESE specimens (Silber and Johnson, 1998).

References
Dear Sir,

Round spermatid injection (ROSI) into oocytes is a recently developed technique of assisted reproduction that can be used for men who fail to produce spermatozoa; using this technique, several human births have been reported (Tesarik et al., 1995, 1996; Vanderzwalmen et al., 1997; Barak et al., 1998). On the other hand, with the current state of the art, the risk of ROSI failure remains high (Amer et al., 1997; Vanderzwalmen et al., 1997).

The Silber et al. article commenting on our opinion article on current problems with spermatic conception (Sousa et al., 1998), is actually a mere repetition of arguments that have been refuted in another recent paper (Tesarik et al., 1998a). These arguments are biased by two major flaws, concerning methodology and interpretation respectively.

The first flaw concerns methodology and relates to the author's unjustified confidence in the value of a simple microscopical observation of native preparations of testicular cells. It appears that Silber et al. are ready to give the label of spermatid only to those cells in which an acrosomal vesicle is visible at that level of observation. Here, of course, they are wrong, because the acrosomal vesicle can be observed only during a limited time period of round spermatid development. As a matter of fact, round spermatids from many patients suffering from complete spermiogenesis failure remain arrested at the Golgi phase of acrosomal development. It is well-known that no acrosomal vesicle can be observed in round spermatids at this stage. This was the reason why, in the original detailed description of the ROSI technique (Tesarik and Mendoza, 1996), we only mentioned the presence of the acrosomal vesicle as one of the characteristics of round spermatids to be detected, certainly not as the decisive one. Notwithstanding, human round spermatids can be identified in the native state by simply respecting the criteria of cell size (approximately that of red blood cells, that usually are numerous in testicular biopsy samples) and by detecting the presence of a round nucleus surrounded by a rim of cytoplasm (distinguishing round spermatids from small lymphocytes, in which the outline of the nucleus cannot be seen) (Tesarik and Mendoza, 1996).

The application of optical systems facilitating the recognition of the acrosomal vesicle, such as the use of DDL phase contrast, is thus only of relative value. The spermatid nucleus and the acrosomal vesicle can be recognized by an experienced worker even with the use of standard Hoffman-contrast optical systems that are currently used in laboratories performing micromanipulation-assisted fertilization. This is demonstrated in Figure 1 of our recent paper (Tesarik et al., 1998a) or in Figure 2 of another recent publication (Vanderzwalmen et al., 1998) in which both structures are clearly visible. Unfortunately, the resolving power of figures in our previous paper (Sousa et al., 1998) has been partly lost during the conversion of the original colour prints to halftones. Even so, only a very inexperienced worker might be able to confuse these cells with Sertoli cell nuclei, simply because the latter are considerably larger than round spermatids, as discussed previously (Tesarik et al., 1998a). Confusion between a Sertoli cell nucleus and a round spermatid is thus definitely no serious obstacle of ROSI.

The tendency for putting too much stress on the detection of the acrosomal vesicle is likely to be at the origin of the inability of some workers, including Silber et al. to identify Golgi-phase round spermatids in the absence of elongated spermatids or spermatozoa in testicular biopsy samples. Interestingly, in the interpretation of Silber et al., this methodological shortcoming has been at the origin of a ‘theory’ that is being defended by the first author for a couple of years against solid arguments showing the contrary, including results from laboratories using advanced techniques of germ cell recognition, such as fluorescent in-situ hybridization (FISH) and