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Letters to the Editor

Origin of leukocytes and their profile in follicular aspirates

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Sir,

In their recent paper, Smith *et al*. (2005) concluded that firstly the presence of leukocytes within follicular aspirates was not directly related to the vessel damage during oocyte harvesting, and secondly, the increase in the relative proportions of monocytes/macrophages compared with polymorphonuclear (PMN) leukocytes observed in several follicular aspirates during their study was indicative of an increase in follicular maturation.

I agree that vessel damage during oocyte harvesting is not the primary source of leukocytes and erythrocytes in human follicular aspirates. Similarly, in our previous study (Akkoyunlu *et al*., 2004), we have observed different subpopulations of leukocytes and erythrocytes between cumulus mass as a consequence of theca rupture during ovulation in mice. Furthermore, after mating, increased amounts of leukocytes were present in pregnant mice, suggesting the uterine leukocytes' infiltration to serve as an immunological barrier and for the phagocytosis of the remaining sperm after a successful fertilization in mice.

I have a criticism of the authors' experimental design regarding the number of samples included in the determination of the relationship between leukocytes and erythrocytes in follicular aspirates: only 13 samples were analysed. The same criticism can be said for the analysis of leukocyte subtypes in a separate group of 14 samples. The results obtained would have been more convincing if all the 27 individual samples had been included in both phases of the experiment.

In addition, when the profiles of the leukocyte subtypes were identified, only CD45 antibody was used for the microscopic observation of follicular aspirates. Instead of just using CD45 (leukocyte common antigen) as a general marker of all the leukocytes, I suggest for a more accurate determination of subtypes, other antibodies such as CD4, CD8 and CD163 should be included in the quantification of the leukocytes.

The authors' speculation about follicular maturation, preliminary or not, should be supported by other parameters such as the simultaneous determination of plasma estradiol levels and ultrasound measurement of the size of the follicles.

References

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Smith MP, Flannery GR, Randle BJ, Jenkins JM and Holmes CH (2005) Leukocyte origin and profile in follicular aspirates at oocyte retrieval. Hum Reprod 20,3526–3531.

Reply: Origin of leukocytes and their profile in follicular aspirates

Sir,

We appreciate Dr Akkoyunlu's interest in our recent article on the origin of leukocytes and their profile in follicular aspirates at oocyte retrieval (Smith *et al*., 2005).

Dr Akkoyunlu draws a comparison between our own study on harvested human follicular aspirates at oocyte retrieval and that of Akkoyunlu *et al*. (2004) on the presence of leukocytes and erythrocytes in the cumulus mass before and after thecal rupture in the mouse. On this basis, it is stated that we have 'concluded that ... the presence of leukocytes and erythrocytes within follicular aspirates was not directly related to the vessel damage during oocyte harvesting'. This is an overstatement: in fact, our conclusion was somewhat more cautious. Erythrocytes do not normally extravasate: their presence in all the samples we examined indicates that a degree of blood vessel damage must have contributed to the composition of these follicular aspirates. Our finding that the numbers of leukocytes and erythrocytes varied independently in the aspirates led us to conclude simply that leukocytes were unlikely to be present solely as a result of vessel damage – we made no generic claim about whether blood vessel damage is the primary source of these cells or not. On the contrary, our study suggests that follicular aspirates are inherently variable in this respect: in some cases, blood vessel damage does not appear to account for all leukocytes present, whereas in others, the numbers of leukocytes originating from damaged vessels may mask any underlying contribution made by other mechanisms such as inflammation.

Our study used two separate groups of follicular aspirates: one to measure erythrocytes and leukocytes and a second independent group to assess leukocyte subtypes. Dr Akkoyunlu is critical of this experimental design, arguing that our study results would have been more convincing had these two groups been combined. In the first place, the collection and analysis of clinical material can impose constraints over and above those involving animal studies, and, in this case, only a limited number of samples were available. In addition, the two different phases of our study required the use of two different protocols for sample preparation. In the first group of aspirates, erythrocytes

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