Technical Report: Mouse Fetal Blood Collection Taking the Best out of the Old Needle-Syringe Method

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Summary

Collection of mice blood is not a common practice due to technical difficulties and the small volumes collected. This constrains many researchers that often choose indirect types of sampling to follow fetal and gestational development, diagnosis and therapy. This study is part of wider studies, in which the above technique was optimized in order to obtain larger volumes.

Timed-pregnant ICR (CD-1®) mice were used. After anesthesia fetuses were rapidly delivered by hysterectomy at gestational days 15, 16, 17 and 18. Large vessels in the fetuses thorax were cut, and blood was collected using several different techniques: a) capillary tubes, b) Microvette® CB 300 (Sarstedt), c) micropipetes, d) different gauge needles and syringe, and e) a hand-modified 20G needle applied to a 1ml/100 IU syringe.

The volumes obtained with the modified 20G needle at 15, 16, 17 and 18 gestational days ranged respectively from: 30 to 45, 50 to 70, 80 to 100, and 120 to 140 µl. The optimization of this technique allows the measurement of biochemical parameters during fetal development and may help to reduce the number of animals used for similar procedures.

Introduction

Collection of mouse blood is not a common practice due to technical difficulties and the small volumes collected. This constrains many researchers that often choose other indirect types of sampling to follow fetal and gestational development, diagnosis and therapy (*Devaskar et al., 1994*). Furthermore, fetal blood contains hematopoietic progenitor stem cells that can be successfully used for cell culture or as an alternative source for related and unrelated bone marrow transplants (*Kernan et al., 1994; Miniero et al., 1995*). However, the results of the fetal transplants depend on the number of nucleated transplanted cells, which in turn depends to some extent on the collection technique (*Gordon and Blackett, 1995*). This study is part of wider studies, in which the above technique was optimized in order to obtain larger volumes.

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Materials & Methods

All procedures involving live animals were conducted in accordance with the National legislation and The Council Of European Convention ETS 123 on the Protection of Animals Used for Experimental and Other Scientific Purposes (*L358/1, November 24, 1986*).

Outbred mice, *Mus musculus*, strain ICR (CD-1®), aged 6 weeks and with conventional microbiologi-

cal status were obtained from Harlan Interfauna Iberica (Barcelona, Spain). The mice were housed in standard colony cages in ventilated cabinets (Tecniplast, Italy), at 22ºC, fed *ad libitum* with tap water and a commercial diet (Panlab, Spain), and exposed to day-night cycles alternatively every 12 h throughout the study period. Mating was performed according to a polygamist regimen (*Broustail, 1967*), in which male and female animals stud together only for a short period of time (not exceeding 16 hours). Timed-pregnant mice (visualization of vaginal plug at day 0) were obtained on days 15 to 18.

Animals were placed alone in an induction chamber with gas input and exhaust output lines. Anesthesia was induced with 5% isoflurane in 100% oxygen with a delivery rate of 5 l/min until loss of righting reflex. After induction animals were moved to a homeothermic blanket and placed in dorsal recumbence. Anesthesia was then maintained with a flux of 1.5 l/min administered with a face mask connected to a coaxial circuit. Fetuses were obtained at gestational days 15, 16, 17 and 18. The whole uterus was removed and placed in a bath with 0.9% NaCl. Direct complementary anesthesia to the fetuses was not necessary, since the time gap between hysterectomy and fetal manipulation (about 7 to 10 min.), allowed death to overcome naturally by anemia or anoxia. Fetuses were then viewed under a magnifying glass (Nikon, Japan), Figure1.

Attempts to collect intra-cardiac blood with 25G needles were unsuccessful, and couldn't be performed on 15 gestational-day fetuses. Large vessels in the fetus' thorax were cut and blood was collect-

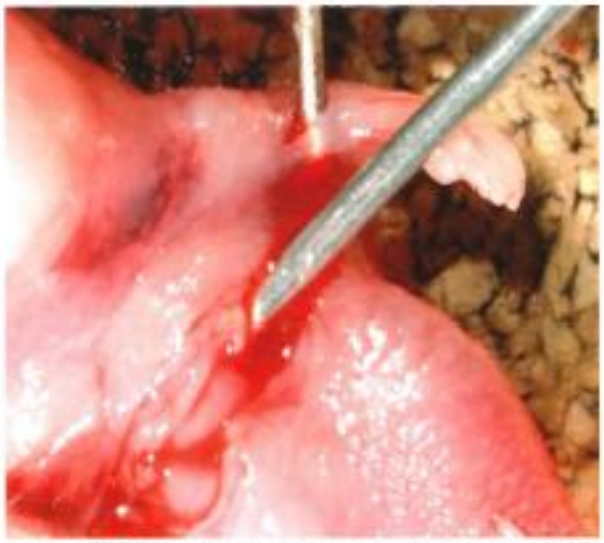

Figure 2. Fetal blood collection with the modified 20G needle, after cutting large vessels in the thorax (17 gestational days).

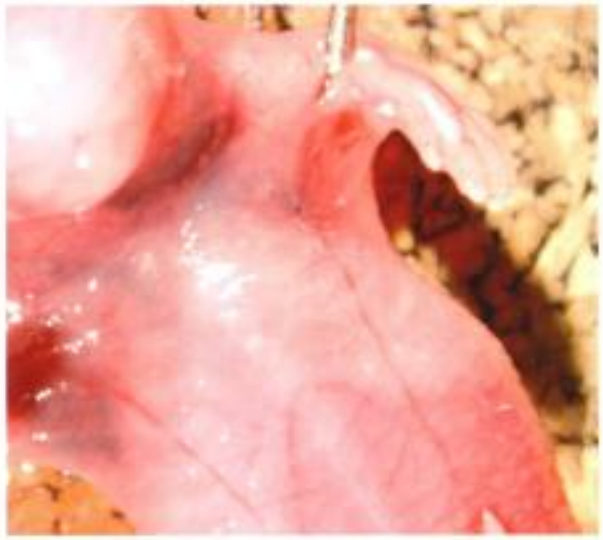

Figure 1. Fetus with 17 gestational days placed under the magnifying glass.

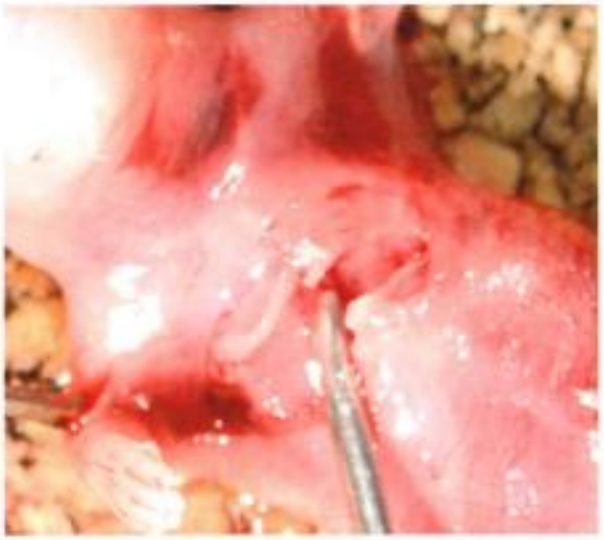

Figure 3. Fetal thorax without blood or clots after blood collection, and without damage to the internal organs (17 gestational days).

ed using several different techniques: a) capillary tubes; b) Microvette® CB 300 (Sarstedt); c) micropipettes, and a series of 25, 21, 20 and 19 gauge needles (Sterican 100, B. Braun, Germany) and syringe; d) a hand-modified 20G needle attached to a 1ml/100 IU syringe (Ominifix 100 solo, B. Braun, Germany) (Fig. 2 and 3). The bevel of the 20G needle was hand-modified to become shorter with the help of a grindstone. It was then smoothed in the same manner to remove its sharp edges (Fig. 4).

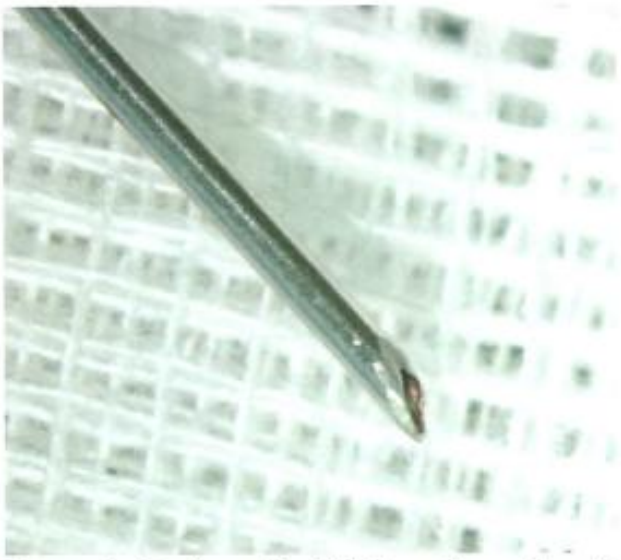

Figure 4. Hand-modified 20G needle made with the help of a grindstone. The bevel is shorter and edge free.

Results & Conclusions

Fetal intra-cardiac blood collection is not a recommend practice due to the small organ dimensions. Our work showed that by cutting large vessels in the fetal thorax sufficient blood volumes were obtained. However, the use of large gauge needles is not recommended, since they have a poor volume/time ratio collection, allowing blood clotting. On the other hand, the thickness and the relatively higher internal diameter of 19G needles render them inappropriate for the use in mice fetuses, since they damage, and tend to suck in, the heart and/or the lungs during blood collection.

Using the modified 20G needle enabled us to collect volumes that ranged from 30 to 45, 50 to 70, 80 to 100 (Fig. 5), and 120 to 140 µl at respectively 15, 16, 17 and 18 gestational days (Table 1). These values represent a prominent increase in each blood volume collection when compared with the other techniques or commercially available systems.

Figure 5. Blood collected at 17 gestational days with the modified needle (100 µl).

Table 1. Blood volume collected (µl per fetus) in the ages under study.

Gestational days	Volume range (µl)	Mean ± (SD)
15 (*n*=113)	30 - 45	40 (5)
16 (*n*=102)	50 - 70	63 (6)
17 (*n*=103)	80 - 100	91 (7)
18 (*n*=101)	120 - 140	133 (7)

SD: standard deviation

The optimization of the fetal blood collection technique provided blood volumes that allow the measurement of several physiological and pathological biochemical parameters. The volumes obtained can provide a good source for stem cell cultures and, as was demonstrated regarding newborn blood (*de La Salle et al., 1996*), a volume that may probably be used for engrafting adult mice without inducing graft-versus-host disease. In summary, mice fetal blood collection can be performed successfully, and the optimization of this technique can contribute to the reduction of the number of animals used for similar procedures. The same methodology could also be applicable to neonates and pups of young ages, yielding better results than other techniques.

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