Brief Communication

Non-surgical Transfer of Cow Eggs

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Despite repeated attempts by many workers to transfer eggs to the cow by non-surgical methods, no success was achieved until the recent report of a single pregnancy by Mutter, Graden & Olds (1964). Subsequently, Sugie (1965) using a technique involving the puncture of the anterior wall of the vagina and the inflation of the uterus with carbon dioxide reported two more successful transfers; one of which however was followed by abortion.

The difficulty inherent in the technique of non-surgical transfer of cow eggs was attributed by Rowson, Lamming & Fry (1953), Harper, Bennett & Rowson (1961) and Bennett & Rowson (1961) to at least two causes (i) uterine infection, and (ii) expulsion of the eggs via the cervix, the two factors acting either singly or in conjunction with each other. Our efforts to overcome these factors remained ineffectual until recently, when using a technique which involves inflation of the uterus with carbon dioxide immediately after deposition of the fertilized eggs in the uterine lumen, we were able to achieve some success.

Before the actual transfer the oestrous cycles of the donor and recipient cows were synchronized by daily intramuscular injections of 50 mg progesterone in arachis oil. Superovulation was induced in the donor animals by a subcutaneous injection of gonadotrophin, either 30,000 i.u. of an anterior pituitary extract (AP) (‘Trovet’, Abbott Laboratories) or 2500 i.u. of pregnant mare’s serum (PMS). The donor cows, thus pre-treated, were slaughtered at 4 to 6 days after the onset of oestrus, and the eggs obtained from the uterus as follows. Immediately after slaughter the reproductive tract was removed and brought back to the laboratory. The oviduct was dissected free of any attached tissue and the ovarian end placed between two pads of gauze soaked in alcohol. This portion of the oviduct was then held by sterile forceps, severed and the residual alcohol washed off with sterile saline. The cervical end of each uterine horn was then seared with a red-hot iron and the seared site punctured with a blunt needle attached to a 20-ml syringe containing sterile bovine blood serum. Each horn was flushed with serum, the flushings passing through the oviduct. The flush fluids were collected in special cups which could be placed directly under the dissecting microscope for immediate examination. Using this technique, we obtained sixty-seven eggs out of a total of 141 ovulations in seven donor cows. Of the sixty-seven eggs thus recovered, forty-eight were found to have been

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fertilized. They were stored at 30° C in cow serum until the time of transfer which was always carried out within 1 hr after egg removal from the donors.

Transfer to the recipient cows was carried out by drawing the eggs (two or three per recipient) together with 0.5 ml of serum into an ordinary insemination pipette. The pipette was introduced through a sterile speculum into the cervical canal of the recipient and passed forward along one uterine horn. After the eggs had been deposited, the pipette was withdrawn until its tip reached the body of the uterus. Carbon dioxide was then introduced via the same pipette until the uterus became fully distended. The gassing equipment comprised two flasks, one containing a few pieces of dry ice was connected to a second one, half-filled with water, which acted as a rough indicator of the rate of flow.

The flask was joined to the applicator nozzle through a T-piece leading off to a rubber balloon which distended as the pressure within the uterus increased; this balloon acted as a safety valve to prevent uterine rupture from too great a pressure within the system. Immediately following gassing, the pipette was withdrawn through the cervix, and, although in a few cases some bubbles of gas escaped, in most the uterus remained distended.

Using the above described technique, material consisting of six cell eggs to early morulae was deposited in altogether fourteen recipients. In six of these where the onset of oestrus of the donor and recipient differed by more than 48 hr, none of the animals became pregnant. In the remaining eight recipients, the cycles were synchronized to ±24 hr with their respective donors; of these eight animals, three became pregnant, but one aborted at 50 days of pregnancy, possibly due to repeated rectal examinations which had been carried out so as to follow embryonic development. The conception rate thus obtained (38%) is admittedly low. To some extent this may have been due to the use of progesterone for the purpose of synchronizing the oestrous cycles; this treatment is known to reduce fertility. The mechanism by which carbon dioxide assists in the establishment of pregnancy remains, as yet, unexplored. It may perhaps depend on a relaxing or anaesthetizing effect on the uterine musculature, which in turn prevents the expulsion of the transferred eggs from the recipient's uterus.

REFERENCES


