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DESCRIPTION OF A SIMPLE METHOD FOR *IN VIVO* FOLLICULAR OOCYTE RECOVERY IN THE RABBIT

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Abstract : A simple and inexpensive folliculocentesis set was developed for *in vivo* recovery of rabbit follicular oocytes. The work focused on evaluating recovery rates and procedure sequels. The folliculocentesis set was composed of two concentric catheters. Briefly, the procedure involves inserting the needle point, attached to the inner catheter, into the follicular cavity; flushing the perfusion medium at the same time, in order to wash the follicular contents. 14 does were used in this experiment. Folliculocentesis was performed 7-9 hours after hCG-treatment (25 IU). The maximum diameter of the intact follicles to be punctured was 1.5 mm. The average operating time was around 14 minutes per doe. Overall, per doe, an average of 10.14 follicles were punctured and 7.0 oocytes were recovered (recovery rate 69%). Does were slaughtered 48 hours after follicular puncture and no sequels were detected at the ovarian level.

RESUME : Description d'une méthode simple de récolte in vivo d'oocytes folliculaires chez le lapin

Un dispositif simple et peu couteux destiné à la récolte in vivo d'oocytes folliculaires a été mis au point. Cette étude permet d'évaluer les taux de récolte et les séquelles dues au procédé. Ce dispositif est constitué de deux cathétères concentriques. En bref le procédé consiste à insérer l'extrémité de l'aiguille fixée au cathétère intérieure dans la cavité folliculaire, et à injecter en même temps le milieu de perfusion afin de laver le contenu folliculaire. 14 lapines ont été utilisées pour cette expérimentation. La récolte folliculaire a été pratiquée 7-9 heures après un traitement par hCG (25 UI). Le diamètre maximum des follicules intacts qui ont été ponctionnés est de 1,5mm. Le temps moyen nécessaire à l'opération est de 14 minutes par lapines. En définitif, un moyenne de 10,14 follicules par lapines ont été ponctionnés et on a enregistré 7,0 oocytes (taux de récolte 69%). Les lapines ont été sacrifiées 48 heures après la ponction folliculaire et aucune séquelles n'ont été détectées au niveau ovarien.

INTRODUCTION

In order to research into *in vitro* oocyte maturation, routine recovery of preovulatory follicular oocytes is necessary. In other commercial species, the ovaries of slaughterhouse's animals are a cheap and abundant source of this kind of oocyte. However, this is not possible in rabbits because the preovulatory follicles are not present at the end of the fattening period (HULOT *et al.*, 1982). In rabbits, the follicular oocytes can only be obtained from healthy, sexually mature, adult does (4.5-5.0 months old at least) (FOX *et al.*, 1964). In these does, routine oocyte recovery takes place *post-mortem* (THIBAULT *et al.*, 1976). Superovulatory treatments (FSH: FOOTE *et al.*, 1992; SEIDEL *et al.*, 1992; PMSG: FOX *et al.*, 1968; GARCIA-XIMENEZ *et al.*, 1990; TANEJA *et al.*, 1990; FUKUNARI *et al.*, 1990) lead to greatly varied individual response, and inferior oocyte quality (FOX *et al.*, 1968; FUKUNARI *et al.*, 1990).

When the resources of healthy, mature does is limited, obtaining follicular oocytes *in vivo* could be an interesting alternative.

Although this procedure may be carried out using superovulatory treatments, repeated applications of such treatments reduce their effectiveness progressively (SEIDEL, 1992).

The present study was made to establish a simple protocol for follicular oocyte recovery from does *in vivo* . An evaluation of recovery effectiveness, as well as possible ovarian surgical damage are also made.

MATERIAL AND METHODS

Animals and general procedure

Fourteen virgin adult New Zealand does (4.5-5.0 months old) were used.

The does were kept in individual cages for twenty days before oocyte recovery.

Because the ovulation (follicular rupture) normally takes place 9:30 to 10 hours after the ovulatory stimulation (THIBAULT *et al.*, 1976) the folliculocentesis was planed about 8 hours after the endovenous injection of an ovulatory hCG dose (25 IU hCG, Coriogan, Ovejero).

Equipment

The folliculocentesis set is made up of a collector tube, a puncture-injection tube and a 50 ml container.

The collector tube is a twenty cm long PVC tube. Its inner diameter is 1.5 mm (this diameter fits the maximum apparent follicular diameter to be punctured).

One end of the collector tube is connected to a sterile container, while the other is cut perpendicular to the tube's length axis. The thin-wall siliconized needle of a sterile, disposable scalp vein infusion set (punction-injection tube), is inserted at 16 mm to the latter end. The needle protrudes 1 mm from the PVC tube.

The punction-injection tube is connected to a syringe (10 ml) which contains perfusion medium.

Procedure

Each animal received an endovenous hCG dose (25 IU, Coriogan, Ovejero).

At 7:15 to 9:15 hours post-hCG treatment, the donor does were anaesthetised by an i.m. injection of 5:1 of ketamine chlorhydrate (ketolar 50 mg/ml., Parke-Davis) prometazine (Phenergan 25 mg/ml., Rhone-Poulenc) solution (1.2 ml/kg body weight), followed 5 min later by i.v. injection of 1.5 ml of the same solution in the marginal ear vein.

Folliculocentesis was carried out by midline ventral laparotomy.

The external follicle surface must to be covered by the collector tube. The needle was pushed over the follicle, until it entered the antral cavity.

At the same time, perfusion medium was injected (0.5 ml/follicle).

The follicular content was washed and swept away through the collector tube to the container.

Table 1: Results of *in vivo* follicular oocyte recovery.

Does	Number pun. follicles	Recovered oocyte number			Post hCG Injection Time	Total time (min)
		Total	With COC	Without COC		
1	9	6	5	1	8h 15m	14
2	13	9	9	0	8h 04m	11
3	10	7	7	0	8h 32m	14
4	8	6	6	0	9h 18m	12
5	8	6	6	0	8h 32m	13
6	6	6	6	0	9h 15m	20
7	12	11	11	0	7h 30m	13
8	5	3	3	0	7h 45m	15
9	13	6	6	0	7h 32m	14
10	11	4	4	0	8h 00m	11
11	11	7	7	0	8h 18m	8
12	14	10	10	0	7h 15m	15
13	11	8	9	1	7h 59m	16
14	11	9	8	1	8h 34m	16
Total	142	98	95	3	----	----
Average	10.14	7.0	6.8	0.2	----	13.7

Number pun. follicles.: Number of punctured follicles in each case.
COC: Cumulus-Oocyte Complex
Total time: Time from laparotomy to put back the does on their cages.

In most cases, the cumulus were visible as they passed along the collector tube.

When folliculocentesis had taken place, the does were sutured and put back in their cages.

The container contents were aliquoted into watch glasses. The number of recovered oocytes was recorded. Oocytes with or without cumulus cells were distinguished because the absence of cumulus means overripe oocytes (intrafollicular ageing).

Other characteristics of the recovered oocytes were not tested, given that this work focuses only on the technical validation of folliculocentesis. Furthermore, the oocyte quality depends on ovarian status more than the folliculocentesis technique.

Forty-eight hours after folliculocentesis, the does were killed by cervical dislocation and the ovarian sequels were evaluated.

RESULTS AND DISCUSSION

At the time of folliculocentesis, ovulation had been induced in only one doe. In this case, follicular aspiration was carried out at 9:15 hours after hCG treatment. Only intact follicles were punctured.

The average number of punctured follicles was 10.14 and the recovered oocytes 7.0, giving a mean recovery rate of 69% (Table 1).

In three cases, oocytes without cumulus were recorded; perhaps some atretic follicles had been punctured.

The time required for the complete operation (from laparotomy to returning the does to their cages) fluctuated from eight minutes to twenty minutes, the average duration was around fourteen minutes.

A follicular haemorrhage occurred only when the follicular wall was damaged excessively.

No damage was observed in the ovaries at 48 hours after folliculocentesis. Moreover the *corpora lutea* appeared to be completely normal.

This procedure was performed only one for each doe so, the efficiency of repeated follicular recovery, performed on the same doe, would be tested.

In the reviewed bibliography we have not found any reference to *in vivo* follicular oocyte recovery in rabbit. In other commercial species, such as sheep (BALDASSARRE *et al.*, 1994) and cattle (ARMSTRONG *et al.*, 1992), normally *in vivo* follicular oocyte recovery is used. This is carried out by either a simple punction-aspiration system or by a punction-intrafollicular washed, double system. The latter is closer to the one we propose, but the catheters are not concentric.

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