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Sex Selection of Mice Embryo by Ericsson Method Using Intra-Peritoneal Insemination

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Summary

The objective of the study was to investigate the effect of Ericsson Albumin Method to separate Y- from X-bearing sperms in mice. The sperms were obtained from caudal epididymis of male mice and prepared by Ericsson method using albumin in two concentrations (8% and 18%) fallowed by intra-peritoneal insemination of female mice. The study showed there was a significant increase ($P<0.05$) in pregnancy rate in female mice that inseminated by sperms were prepared by Ericsson method. Also, the results demonstrated that Ericsson method was found (75%) effective for male gender selection compared to conventional method (51.25%) without separation of Y- from X-bearing sperms.

إختيار جنس الجنين في الفئران بطريقة إيركسون بحقن النطف عبر غشاء البريتون

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الخلاصة

أجريت الدراسة بهدف بحث تأثير إستخدام طريقة إيركسون في فصل النطف الذكرية عن الانثوية في الفئران باستخدام الالبومين وإجراء الإخصاب بطريقة حقن النطف عبر غشاء البريتون. تم الحصول على النطف من ذيل البربخ لذكور الفئران وإجراء عملية التنشيط ثم إستخدمت طريقة إيركسون لفصل النطف الذكرية عن الانثوية باستخدام مادة الالبومين بتركيزين (8% و 18%) ثم إجراء الاخصاب بحقن إناث الفئران بطريقة حقن النطف عبر غشاء البريتون. أظهرت النتائج وجود زيادة معنوية ($P <0.05$) في معدل الحمل عند الفئران الملقحة بالنطف المنشطة بطريقة إيركسون. ووجود زيادة معنوية في معدل المواليد الذكور(75%) بإستخدام الطريقة ذاتها عند مقارنتها مع معدل الذكور (51,25%) عند مثيلاتها من الفئران التي حقنت بالنطف المنشطة بالطريقة الاعتيادية.

Introduction

Sex selection, also known as gender selection, has attracted great interest and controversy over the years. Selection of gender by separating X- from Y-bearing sperm before semen is used in artificial insemination could give the choice of offspring sex (1).

Sperm carry either an X chromosome or a Y chromosome; all eggs carry an X chromosome. If an egg is fertilized by an X-bearing sperm, the offspring will be female, while a Y-bearing sperm will produce a male (2).

In most mammalian, including humans, the ratio of males to females is about 50:50, because the determination of sex, or gender, takes place when a sperm fertilizes an egg, preselecting of gender by selecting the sperm that fertilize eggs must be done before the sperm are used for insemination (1).

Ericsson Technique was developed in the 1970s, Sperm sorting aims to produce a sample with a higher proportion of X- or Y-bearing sperm; this increases the chance of conceiving of the preferred sex (3).

Sex selection performed for medical reasons to avoid sex-linked diseases such as hemophilia and Duchenne's muscular dystrophy (4). Research dealing with sex predetermination in animal, to provide the producer with faster genetic progress and greater flexibility, Gender of animal offspring is important to livestock producers, because the dairy farmer has little use for most bull calves, the use of sexed semen to produce only females would make milk production more efficient (5). In beef cattle and sheep breeds, the male grows at a faster rate than the female and hence is preferred for meat production (6).

In addition, the ability to specify male or female offspring should shorten the time required for genetic improvements, since desirable traits are often associated with one or the other parent (7). Planning the sex of cattle offspring is already practiced on a limited basis, this procedure consists of removing embryos from the cow, identifying their potential gender, and re-implanting
only those of the desired gender (5). However, an ability to separate sperm into male-producing and female-producing groups before they are used for artificial insemination could enhance the overall value of offspring produced by embryo transfer (1).

Materials and Methods

1. Detection of Estrous Cycle

There were 80 mature mice (Balb/C St Can BR Strain) 8-12 weeks old and 25-35g weight, were detected for stages of estrous cycle and reported using vaginal smears. The smears were performed daily between 8:00 Am. and 1:00 pm.

A loop was sterilized by burner and immersed in sterile normal saline, then inserted into the vagina to take a smear from vaginal wall by rolling the loop gently. The smear was spread on a clean slide and stained with 1% methylene blue for 3-5 minutes. Stained smears were then flushed with tap water. The slides were air dried and examined under the light microscope at (400×) to determine estrous cycle (8) (Figure 1).

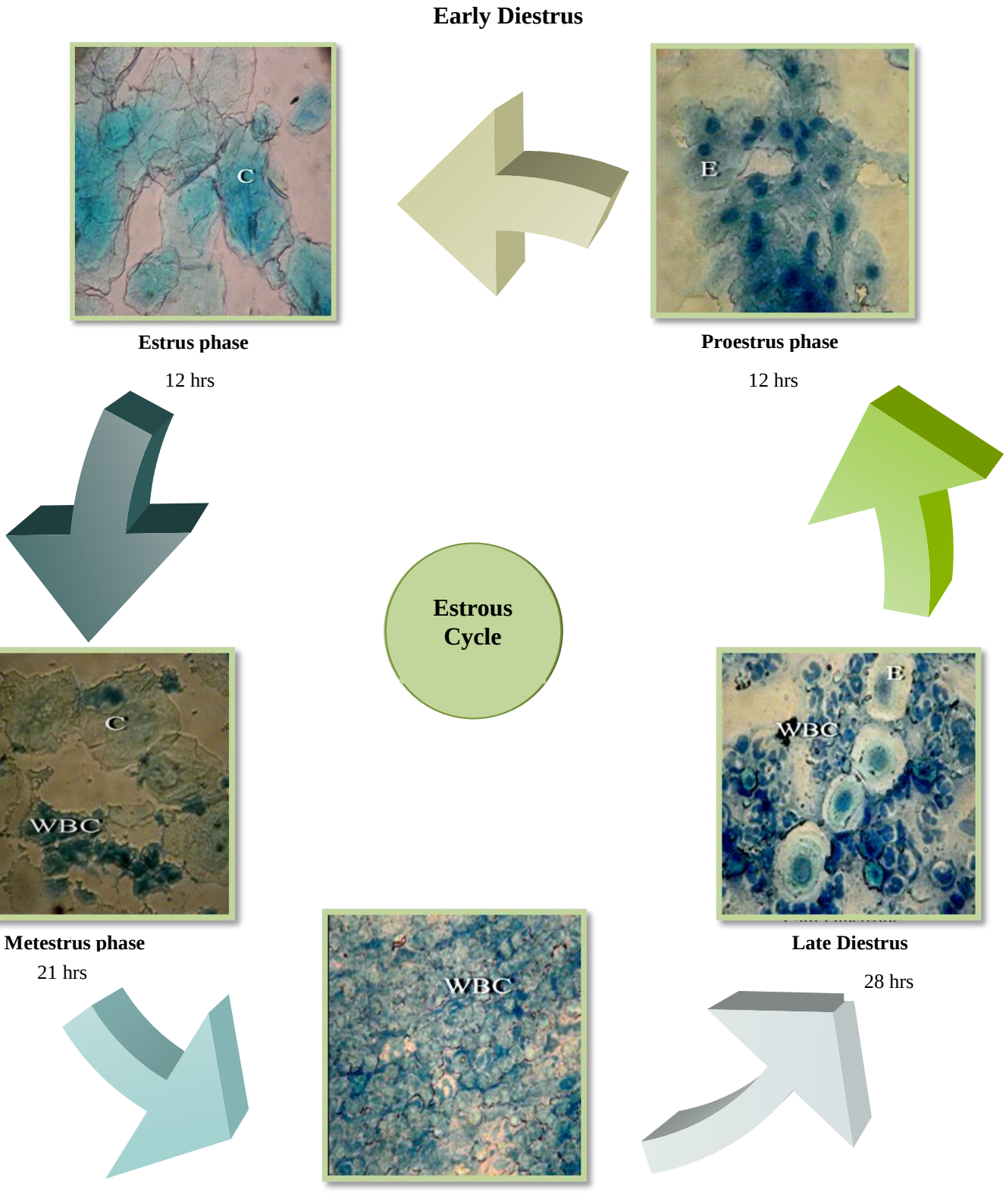

Figure (1): The Estrous cycle in mice
C: Cornified Cell. E: Epithelial Cell. W: White Blood

2. Sperm Collection

1- Sacrificing mature male mice 6-12 weeks old approximately 2 hours before insemination.
2- The caudal epididymis was isolated (Figures 2and 3) and placing on a transfer dish with 1 ml of PBS (phosphate buffer solution).
3- The epididymis content were squeezed out by making 5-7 slashes with 29-gauge needle syringe and allows the sperms to swim-out (Figure 4) and residual caudal tissue was discarded (9).

3. *In Vitro* Sperms Activation Technique

The spermatozoa suspensions were incubated at 37 ºC in 5%CO_2 at least for 30 minutes, using Direct Activation Technique, the caudal epididymis sperms were allowed to swim-up through 30 minutes. This technique for sperm activation was characterized by direct effect of culture medium on sperm parameters. Then the sperms were counted and used to inseminate the control group (8).

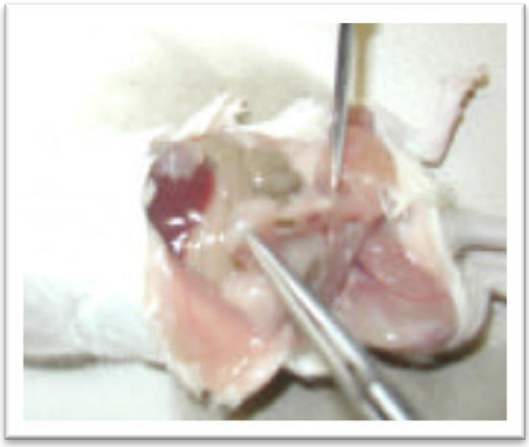

Figure 2: Finding the Caudal Epididymis

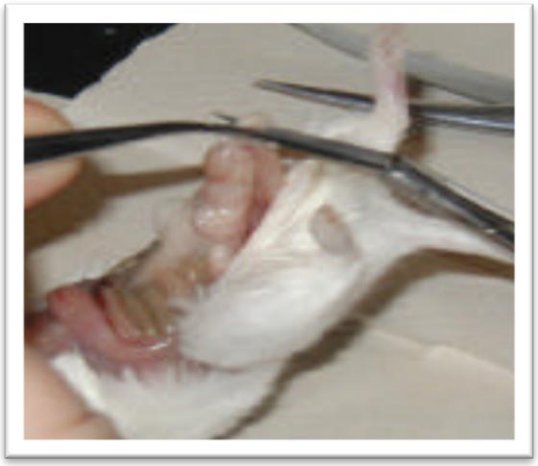

Figure 3: Cutting out the Caudal Epididymis

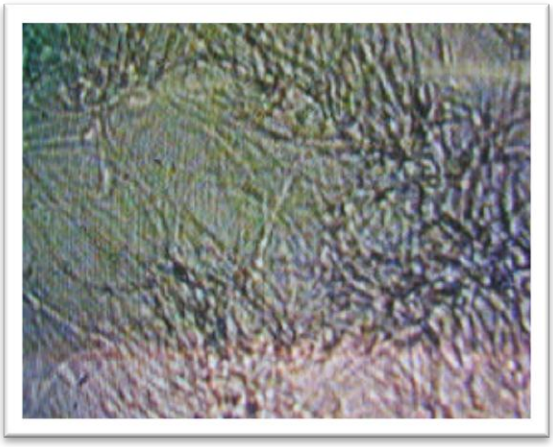

Figure 4: Mice sperms under microscope

4. Sperm Preparation by the Ericsson Albumin Method

Human serum albumin (HSA) was layered into a column of increasing thickness, here of 8% and 18%. Then the spermatozoa suspension was layered on top of the albumin column (Figure 5).

The column was allowed to stand for 1 hour, allowing sperm to penetrate the albumin.

The top layer was discarded, and the column was allowed to stand for another 30 minutes. Then the next layer (originally the middle layer) was discarded.
The remaining bottom layer was centrifuged at 3000 (rPM) for 10 minutes, and the sediment at the bottom was retained and prepared for inseminated the treated group (3).

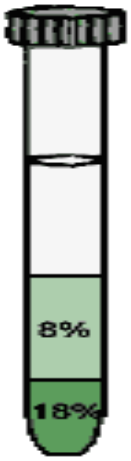

Figure 5: Mice sperms preparation by the Ericsson Albumin Method.

5. Intra-Peritoneal Insemination.

Intra-peritoneal Insemination (IPI) was performed during estrous phase. Spermatozoa concentrations of $2x10^6$ were transferred intra-peritoneal at the right side of ovarian duct (9).

Results

The pregnancy rate was gained by dividing the number of pregnant mice on the total number of inseminated mice.
There were 40 female mice were inseminated intraperitoneally by the sperms were prepared by Ericsson Albumin Method (treated group), the pregnancy rate were 77.5% (31 pregnant mice), while the pregnancy rate in control group was 62.5% (25 pregnant mice out of 40 inseminated mice intraperitoneally by the sperm were prepared by Direct Activation Method). There a was significant ($P<0.05$) increase in pregnancy rate between two groups as shown in table (1).
In treated group, the total numbers of births were 200 of them, 150 (75%) males, and 50(25%) were females. While in control group the total numbers of births were 160, there were 82(51.25%) males, and 78(48.75%) females. There was a significant ($P<0.05$) increase in the number of males by using Albumin Method, while there was a significant decrease ($P<0.05$) in the number of females in treated group compared to control group as shown in table (1).

Table 1: Effect of Ericsson Albumin Method on Pregnancy Rate and Sex Selection in Mice.

Pregnancy Rate		**P-Value**	**Total No.of Births**	**No.of Males**	**P-Value**	**No.of Females**	**P-Value**
Treated Group	**31/40 (77.5%)**	**S**	**200**	**150 (75%)**	**S**	**50 (25%)**	**S**
Control Group	**25/40 (62.5%)**		**160**	**82 (51.25%)**		**78 (48.75%)**	

Discussion

The results were showed significant increases in pregnancy rate and sex selection when the females mice were inseminated by the sperms were prepared by Ericsson Albumin

Method (table1). Many factors might interfere with this observation. Regarding sperm side, the Ericsson Albumin Method has a positive effect on sperm motility and forward movement, which is an essential prerequisite for fertilization and conventional methods of assisted reproduction (10).

It has been suggested that sperm spinning techniques for gender selection are only minor variations on a standard procedure that is used to prepare sperm for assisted reproduction such as intrauterine insemination (IUI) or *in vitro* fertilization (IVF) (11), there are several purposes of these techniques such as; separate the sperm cells from the semen, or fluid. During normal intercourse, only the sperm cells, not the seminal fluid, enter the uterus; semen in the uterus can cause contractions which prevent fertilization, or even a dangerous allergic reaction (12).

Ericsson Albumin Method is based on the assumption that Y-sperm swim faster than X-sperms, sperms are placed in a test tube atop a "column" of increasingly thicker layers of albumin (8% and 18%), and allowed to swim down into the solution, after a certain time period has elapsed, only the fastest sperms should have been able to penetrate to the bottom layer (3). Furthermore in Ericsson method the Human Serum Albumin (HSA), a sticky protein solution so that concentrate healthy, motile sperms, filter out abnormal and immotile sperms cells and debris, such as dead sperms cells and bacteria that may impede fertilization and appears to select population of sperms with normal morphology and high motility (2), the resulting sperms sample is much more likely to result in a successful pregnancy than if unprocessed sperms were used for insemination (table1), the procedure is sometimes referred to as "sperms separation" or "sperms sorting", because it separates the best sperms from low quality sperms (13), since albumin method, is standard procedures for IUI and IVF, it is considered safe (14), attempted gender selection is either an additional or modified step to these standard techniques, this finding is in agreement with (15), who emphasized that the only parameter that could predict treatment outcome was the percentage of motile spermatozoa after appropriate sperms preparation (15).

Researchers began reporting experiments of spinning sperm in various types of solutions and several initially reported promising success in creating X or Y-enriched samples (13). However, it would later turn out that the method then used to test the resulting sperm samples, called quinacrine staining, was flawed, with the later development of a reliable method to measure the X:Y ratio of sperms, called "fluorescence in situ hybridization" (FISH), repeated experiments showed that none of the proposed sperm spinning methods actually altered X:Y ratio of sperm (17).

Experiments using the Ericsson method have shown that it does not create X or Y-enriched sperm samples; the results remain 50/50 is it possible that sperms of one gender are somehow given a functional advantage, or the other gender somehow inactivated or incapacitated (18). Sperms being separated on the basis of their swimming ability, depending on whether a female or male is desired, a different fraction of the sperm is recovered and used to artificially inseminate (19).

It is concluded from the present work that the Ericsson Albumin Method was founded 75% effective for male gender selection, even though sperms X: Y ratio was unchanged after the procedure, furthermore the pregnancy rate significantly more than using the Direct Activation Technique. This result can be utilized for other mammalian Assisted Reproductive Technologies Programs.

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