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## Artificial Insemination in Rabbits

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**SUMMARY.** *The success of artificial insemination (AI) technique in rabbits depends on various factors that act in a complex way. These include type of semen, supplementation of semen with some stimulators, method of inducing ovulation and doe status.*

*Rabbit semen has been successfully stored on liquid state for short periods of time (6h for fresh and 48h for refrigerated) without serious loss of its fertilizing ability. However, there are few recent information on the comparative values of different diluents. The efficiency of AI with frozen semen did not reach the level of fresh semen. This is because rabbit spermatozoa are relatively sensitive to cryoprotectants added before freezing. Cryoprotectants containing hydroxyl groups are relatively less effective than those containing amide or methyl groups. Supplementation of rabbit semen with PGE<sub>2</sub> improved fertility. However, PGF<sub>2</sub>α produced a detrimental effect. Insemination with cooled rabbit semen supplemented with 5 mM theophylline was as effective as fresh semen and improved the kindling rate by about 22.6% when compared to the non-supplemented cooled semen. The development of a practical method for induction of ovulation repeatedly with gonadotropin releasing hormone without antigenicity have led to a commercial application of its use. Conception rate of the virgin doe is higher compared to that of the primiparous and multiparous animals. Younger animals are shown to be less fertile than those of 1.0 - 1.5 years, but conception rate decreased in more older animals. Conception rate in lactating does is lower than in dried ones. Does with high live weight also have high conception rates. However, further studies should be carried out before final conclusions can be drawn.*

**Key words:** *type of semen, c-AMP stimulators, ovulation, doe status.*

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### Introduction

AI has been used in research and laboratory procedures for many years in rabbits. Recently, techniques have been improved and refined making a simple, useable, predictable tool for controlled breeding programmes, reproduction studies and defined herd development (Heidbrink *et al.*, 1979; Adams, 1981; Tawfeek and El-Gaafary, 1991 and Facchin, 1992).

Interest in AI is increasing in all the countries where intensive rabbit raising is practiced. However, large differences in conception rate are commonly obtained even under the same conditions.

The purpose of this chapter is to review some of the factors which affect fertility following artificial insemination in rabbits.

## Some factors Affecting Fertility (Conception Rate) Following AI

### 1. TYPE OF SEMEN

#### a. Liquid semen

Rabbit semen has been successfully stored for short periods of time, without serious loss of its fertilizing ability, when using diluents based on either tris or citrate. However, there are surprisingly few recent information on the comparative values of different diluents, inspite of the fact, that a good extender gives maximum fertility with a minimum number of spermatozoa. Pmytko and Andreeva (1973) and Shi (1983) showed that citrate-based diluent was a good media for maintaining the fertilizing ability of rabbit spermatozoa during storage on liquid state. However, Foote (1972) Hahn and Remmers (1974) and Bellotti (1986) reported that tris-based diluent had been also successfully used for preservtion of rabbit semen. According to our laboratory work (El-Gaafary and Ahmed, 1989), citrate - based diluent was found to be better for maintaining motility and acrosome integrity of rabbit spermatozoa during incubation at 37°C for 6 hours and storage at 5°C for two days as compared to tris-based diluent. The kindling rates were 87.5 and 75.0% following insemination of rabbits with semen extended in citrate- and tris-based diluents, respectively. The composition of the two diluents were

as follows: (i) Tris-based diluent: 250 mM tris, 69.4 mM fructose, 79.7 mM citric acid and 20% egg yolk. (ii) Citrate-based diluent: 12.8 mM sodium citrate, 332.2 mM glucose and 20% egg yolk. Almost all the inseminations were done with fresh or refrigerated semen stored for a maximum of 6 and 48 hours, respectively.

#### b. Frozen semen

Several studies have shown that rabbit spermatozoa can be frozen with various degrees of success. However, the fertilizing ability of the sperm is commonly reduced following storage in liquid nitrogen at -196°C (Wales and O'Shea, 1968 and Stranzinger *et al.*, 1971), although some workers (Andrieu and Courot, 1976; Hanada and Nagase, 1980 and Chen *et al.*, 1989b) obtained rather good results.

Deep freezing of rabbit semen is the main impediment to diffusion of large scale AI. The reason is that rabbit semen respond differently than in some other species. Rabbit spermatozoa are very susceptible to damages caused by hypertonic solutions and are relatively sensitive to cryoprotectants containing hydroxyl groups such as glycerol (Chen *et al.*, 1989 a and Castellini *et al.*, 1992), although they are less sensitive to rapid cooling (from 30 to 0°C) than in bull and ram spermatozoa (Hanada and Nagase, 1980). Despite of the numerous attempts, it has not yet

found an effective nontoxic cryoprotective agent, since all the tested substances showed negative effects on sperm survival, motility and acrosome integrity, although cryoprotectants containing amides or methyl groups have been recommended (Hanada and Nagase, 1980 and Castellini *et al.*, 1992).

The most widely used cryoprotectants are glycerol and dimethylsulphoxide (DMSO). The protective effect of such cryoprotectants is largely attributed to their "salt buffering capacity" which minimizes electrolytic damage as the water freezes out (Graham, 1976). Hanada and Nagase (1980) investigated the effectiveness of eleven substances and reported that spermatozoa maintained good motility at 20°C in hypertonic solutions of dimethylsulphoxide, trimethylene glycol, acetamide and ethylene glycol suggesting that these compounds freely permeated the cell membrane and were relatively nontoxic. In contrast, the same authors claimed that sperm survival was low in hypertonic solution of formamide, propionamide and dimethyl formamide. In a subsequent study, the best recovery rates post-thawing (53.8% motility, 44.5% live spermatozoa and 70.4% normal acrosomes) were obtained when using DMSO without glycerol or acetamide (Castellini *et al.*, 1992). Inclusion of 8 % DMSO in the diluent was more effective in protection of rabbit spermatozoa during freezing than did

glycerol (EL-Gaafary *et al.*, 1993). A medium contained 8% DMSO plus 2% glycerol was the best for protection of rabbit semen during freezing and post-thawing, since it maintained the highest percentages of sperm motility, freezability and normal acrosomes. A kindling rate of 42.9% was obtained, however, this result is still rather low.

In conclusion, the efficiency of AI with frozen semen in rabbits did not reach the level of fresh semen. Therefore, it is necessary to test other diluents, evaluate other cryoprotectants and/or their combinations and improve the freezing technique itself.

## 2. Semen supplementation

Supplementation of semen with prostaglandins (PGs) has received attention as a potential promotor of fertility through improving sperm transport and/or survival in female genitalia (Chang *et al.*, 1973 and Edqvist *et al.*, 1975). The mechanism by which seminal PGs stimulate sperm motility may involve an enhancement of adenylate cyclase and a subsequent rise in c-AMP levels (Aitken and Kelly, 1985). A positive relationship between intracellular c-AMP levels and sperm motility was reported (Tash and Means, 1983).

Addition of PGE<sub>2</sub> to rabbit semen caused an increase infertility (Alvarino and Rebollar, 1991). This was a result of increased uterine motility that moved the sperm more

rapidly to the site of fertilization. However, inactivation of PGs in rabbit semen with prostaglandin - 15-hydroxy- dehydrogenase before insemination produced a reduction in fertilization rate (Schelegel *et al.*, 1983). El-Gaafary (1989) and Alvarino and Rebollar (1991) also indicated that supplementation of rabbit semen with  $\text{PGF}_{2\alpha}$  depressed sperm motility and kindling rate (Table 2). The authors suggested that the negative effect might be a result of changes in the sperm cell membrane permeability, resulting of the release of the intracellular enzymes. El-Gaafary *et al.* (1991) emphasized that addition of more than 300mg/ ml  $\text{PGF}_{2\alpha}$  to the rabbit semen resulted in an increase in the number of sperm with damaged acrosome and an increased release of the intracellular sperm enzymes such as glutamic - oxalacetic transaminase, glutamic-pyruvic transaminase and lactate dehydrogenase into the extracellular medium.

From another point of view, addition of phosphodiesterase inhibitors which prevent the breakdown of cyclic 3, 5-adenosine monophosphate such as caffeine and theophylline markedly increased respiration and maintained motility of bull and ram spermatozoa (El-Gaafary, 1987 and El-Gaafary *et al.*, 1990). Supplementation of cooled rabbit semen with 5 mM of each of either caffeine or theophylline increased sperm motility without any deleterious effect on cell membrane

integrity (El-Gaafary, 1994). Moreover, insemination of rabbits with cooled semen supplemented with 5 mM theophylline was as effective as fresh semen and improved the kindling rate by about 22.6% when compared to the non-supplemented semen (Table 3). Further studies should be directed towards finding the types and optimum levels of such stimulators that give the best fertility results.

### 3. Induction of ovulation

Ovulation in rabbits is normally induced as a result of mating and takes place after mating with 10-13 hours. Therefore, an artificially inseminated doe must be stimulated to ovulate with either a vasectomized buck, mechanical stimulation or an injection with a hormone.

The use of a vasectomized buck is not always successful and may contribute to the spread of venereal disease. In addition, as in natural mating, the buck may perform a complete copulatory act yet, it is not certain that ovulation will follow (Heidbrink *et al.*, 1979). The mechanical stimulation of the vagina can induce ovulation, but the outcome is usually quite random (Nieto Vazquez, 1984). The injection of luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) can result in a failure of the doe to ovulate due to antibody formation even though the doe had a positive conception

**Table 1. Some fertility results of frozen rabbit semen.**

Authors	Diluent	Gryoprot- ectant	Type of semen packaging	Fertility (%)
Stranzinger et al. (1971)	Tris-based	15% DMSO + 6% glycerol	Pellet	47.2
	Tris-based	15% DMSO + 6% glycerol	Tubing	33.0
	Tris-based	15% DMSO + 6% glycerol	Straws	18.8
Andriew and Courot (1976)	Tris-based	15% DMSO + 6% glycerol	Straws	69.0
	Tris-based	15% DMSO + 1.3% glycerol	Straws	83.0
Hanada and Nagase (1980)	Glucos-based	1 M DMSO	Pellet	93.0
		1M acetmide	Pellet	88.0
		1M lactamide	Pellet	73.0

**Table 2. Fertility of does inseminated with semen supplemented with PGF<sub>2α</sub> (from El-Gaafary, 1989).**

Treatment	No. of does inseminated	Does kindled	
		(No.)	(%)
Fresh diluted semen	16	13	81.3
Fresh diluted semen + 600 µg PGF <sub>2α</sub>	12	6	50.0

**Table 3. Fertility of fresh, cooled and cooled rabbit semen supplemented with theophylline (from El-Gaafary, 1994).**

Type of semen	No. of does inseminated	No. of does kindled (%)
Fresh diluted semen	17	12 (70.6) <sup>a</sup>
Cooled semen	12	5 (41.7) <sup>b</sup>
Cooled semen + Theophylline	14	9 (64.3) <sup>ab</sup>

Values with different letters, differ significantly (P < 0.05).

response for an initial two or three AI breeding services (Adams, 1972). An alternative treatment now available in the form of synthetic gonadotrophin releasing hormone (GnRH) that can repeatedly injected intramuscularly without eliciting antibody formation (Adams, 1981), has overcome this problem. Injection with GnRH should be given within  $\pm 2$  hours of insemination. It can be given as long as 4 hours before or 10 hours after insemination without affecting the fertility results (Adams, 1976).

#### 4- Doe status

##### A. AGE OF DOE

There is a negative correlation between age of doe and conception rate (Table 4). The virgin does (4-5 months of age) usually show excellent conception rate (Sinkovics *et al.*, 1983). The non-kindled (nullipara) does showed high conception rate (92.0%), while the kindled does (primipara and/or multipara) achieved only 67.1% (Szendro and Biro-Nemeth, 1991). However, some studies showed that conception rate was low when insemination of does was carried out during the first lactation following the first parturition as compared to multiparous females (Bourdillon *et al.*, 1992), and the highest conception rates were obtained with does in fourth parity and inseminated directly after kindling (Lange and Schlolout, 1988).

##### B. TIME OF RE-BREEDING

It is difficult to predict the optimal time for insemination (Table 5) during the suckling period due to the effects of each of hormone regulation during lactation period and the interval between two successive parturitions.

The conception rate was found to be low during the first 15 days after kindling (19.0%), good between 16-40 days (72.3%) and the best after weaning (83.8%) (Szendro *et al.*, 1992). Particularly, differences of 11.8% in conception rate between does inseminated before and after weaning were reported (Szendro and Biro-Nemeth, 1991). However, conflicting results (Paufler *et al.*, 1979) showed that the highest conception rate (77.8%) was obtained by insemination on day 2 when inseminating on days 1, 2, 3, and 4 after parturition. Insemination of does after a short parturition - AI interval, was accompanied with low conception rate, while does inseminated later (day 21 of lactation) produced high conception and prolificacy rates (Reboller *et al.*, 1992). Higher fertility rates were obtained following AI among dried females than among the nursing ones (Bourdillon *et al.*, 1992), although Szendro and Biro-Nemeth (1991) found that conception rate of does inseminated after 60 days of parturition was commonly low due to

**Table 4. Effect of the age of doe on conception rate (from Szendro and Biro-Nemeth, 199).**

Age of doe (Months)	Number of inseminations	Number of kindlings	Conception rate (%)
4-5	63	57	90.5
6-7	113	82	72.6
8-9	81	60	74.1
10-13	88	70	80.0
14-17	65	56	86.1
18-25	45	37	82.2
First conception within 6-7 months	25	23	92.0
Soon kindled	88	59	67.1
Total	455	362	79.6

**Table 5. The effect of time re-breeding on conception rate (from Szendro et al., 1992)**

Re-breeding (days after kindling)	Number of inseminations	Number of kindlings	Conception rate (%)
Before weaning			
1-10	7	2	26.6
11-15	35	6	17.1
16-20	101	72	71.3
21-25	65	46	70.8
26-30	25	14	56.0
31-40	127	98	77.2
1-40	360	238	66.1
After weaning			
41-60	102	88	86.3
above 60	89	72	80.9
40 <	191	160	83.8

**Table 6. Effect of the body weight of does on conception rate (from Szendro and Biro-Nemeth, 1991).**

Body weight of doe (kg) at previous kindling	Number of inseminations	Number of kindlings	Conception rate (%)
Under 3.50	27	21	77.8
3.51-4.00	134	92	68.7
4.01-4.50	144	115	79.9
Above 4.50	38	35	92.1
Total	343	263	76.7



some of the does either failed to show estrus or were barren.

In conclusion, it could be stated that, although does mated early before weaning show low conception rate, but the advantage remain that 65-75% of the does could be pregnant before weaning which increases the profit.

### C. VULVA COLOUR

In natural mating, there is no justification for observation of vulva colour since females accepting a male are receptive in any case. Few authors studied the relationship between vulva colour and fertility, with the aim of predicting receptivity for AI purposes. Receptive does show about 20% higher conception rate than in those unwilling to mate (Adams, 1981). In nulliparous females which are generally highly receptive, fertility rates are fairly similar regardless of vulva colour. In multiparous does, fertility is higher when vulva colour is pink or red (Theau-Clement and Roustan, 1992). Improvement is about 10% in fertility when inseminating only females presenting red, turgescient pink or turgescient purple vulva (Roca *et al.*, 1986). The number of does kindled following AI is significantly higher when the vulva colour is red (55.2%) or pink (51.5%) than when the vulva colour is white (33.3%) (Rashwan and El-Gaafary, 1992). However, failure in does with pale or white vulva to conceive is well known even after natural mating

(Diaz *et al.*, 1987). In general, it may be concluded that fertile does are estimated to be less than 30% with white vulva and 70% with red vulva.

### D. BODY WEIGHT

Definite relationships between body weight and conception rate were reported (Table 6). The body weight of the does at the previous kindling does not influence the conception rate until 4.5 kg, but above this weight, the conception rate increases by about 15 - 20% (Szendro *et al.*, 1992). Particularly, does with 3.5-4.0 kg live weight have average conception rate of 68.7%, whereas, it is 92.1% for those above 4.5 kg (Szendro and Biro-Nemeht, 1991).

### E. SEASON

It was shown that season of the year affected both natural mating and artificial insemination (Sinkovics and Haut, 1988; Valentini *et al.*, 1988 and Ahmed *et al.*, 1991), although according to Sinkovics and Haut (1988), seasonal fluctuations were lower for artificial insemination than for natural mating. Schlolaut *et al.* (1981) obtained good results with AI in summer and fall in Germany when inseminations were carried out immediately after kindling. In this case, very good environmental and feeding conditions were available. In more recent work, Szendro and Biro-Nemeth (1991) and Szendro *et al.* (1992) found that the highest

conception rate was achieved during April and May (91.9%) and the lowest was during August and September (61.1%), in Hungary. The seasonal fluctuations were lower for does inseminated after weaning as compared to those inseminated before weaning.

## Conclusion

Progress in application of artificial insemination in rabbits has been achieved during the last decade. The results obtained using liquid semen could be considered satisfactory and encouraging. However, the use of frozen semen that could give the final input at AI diffusion on a commercial scale is still limited to research and experimental purposes. This is due to lack of knowledge and application in the following topics (a) formulation and preparation of specific cryoprotective dilutents and (b) standardization of a freezing and thawing programmes. Supplementation of buck semen with cyclic-AMP stimulators such as theophylline improved sperm motility and fertility and this may hold promise with frozen semen. The development of practical methods for induction of ovulation repeatedly using synthetic gonadotropin releasing hormone without antibody formation have led to increase of its application. Relationships between artificial insemination and doe status (age of doe, time of re-breeding, colour of vulva, body weight and season) have

been reported, however, further investigations under different environmental conditions are needed to confirm these results.

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