REPRODUCTIVE PERFORMANCE OF DOES UNDER ARTIFICIAL INSEMINATION.USE OF DEEP FROZEN RABBIT SEMEN

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Abstract - Data recorded, on 381 nulliparous (non lactating) and, 42 days later, on 144 primiparous (lactating) does were analysed. The does were inseminated with frozen semen kept during 5 years in liquid nitrogen ; 525 straws from 33 ejaculates from 12 sires have been used. The straws (11 at least) of one ejaculate have been equably spread over the season, the receptivity and the lactating status of the does. Fertility, 0.66 on average, is influenced by receptivity (receptives : 0.79, non receptives : 0.20) and the lactating status (non lactant : 0.64, lactant : 0.36). Because of the low fertility of the non receptive does, the reproductive performance has been analysed in receptive does only. The litter sizes at birth (9.1 total young born, 8.4 born alive) and at weaning (7.9 weaned) are influenced by the male father of the litter and the lactating status of the does. The male only, influences the mean weight of the young rabbit at weaning. The individual motility and the percentage of motile cells had been measured before freezing and after thawing ; no relationship has been observed between these data. Moreover, no relationships have been observed between any of the features of the sperm and reproductive performance.
It is concluded that, once more, receptivity is the major factor in reproductive performance. It is also concluded that, on receptive does, frozen semen kept during 5 years gives good reproductive performance.

INTRODUCTION

Frozen semen would permit to completely dissociate in time and in space semen collection from its use, allowing a better valorization of the work of selection and dissemination of genetic improvement. Satisfactory storage of rabbit semen has not yet been reported on a large scale. Furthermore, the reproduction performance of the does depends largely on the physiological status of the does (THEAU-CLEMENT and ROUSTAN, 1992), combination of sexual receptivity and lactating status, at insemination time.

Nulliparous does are generally receptive and consequently have good fertility but litter size are quite small. Primiparous does have poorer fertility but higher litter size than nulliparous (POUJARDIEU and THEAU-CLEMENT, 1995) leading them to low productivity. So, this model has been chosen to study the relationships between the biological parameters of rabbit semen, before and after freezing, and reproduction and litter performance, taking into account the physiological status of the does.

MATERIAL AND METHODS

Data have been recorded at the INRA Experimental Station, Le Magneraud.
Five hundred twenty five straws made from 33 ejaculates (27 double and 6 single) of 12 Hyplus bucks were used. The semen was deep frozen 5 years prior to this experiment, using ANDRIEU and COUROT (1977) technique, the dilution was constant (1 : 7). So, the number of spermatozoa inseminated varied. The bucks were collected once a week, twice a day in a space of 15 mn; the 2 ejaculates of a same buck were mixed.

Animals

Three hundred and eighty one INRA A1067 does, aged between 18 and 21 weeks and weighing at least 3.2 kg were inseminated. A first group of 226 nulliparous does were inseminated on July 1994 and inseminated again six weeks later, only if they suckled at least one pup. A second group of 155 nulliparous does were inseminated only once on January 1995. So strictly, only nulliparous and primiparous does were used in this experiment.

Experimental design

From 10 weeks of age up to a week before the insemination the does were under a 8L : 16D photoperiod ; and then 16L : 8D for the duration of the experiment (THEAU-CLEMENT *et al.*, 1990). Primiparous were injected 48h before insemination, with 25 UI of PMSG (SANOFI). Prior to insemination, does receptivity was tested (lordosis posture) in the presence of a buck rabbit. This allowed us to know the physiological status of the does : non lactating (nulliparous) receptive does (Al-R+), non lactating (nulliparous) non receptive does (Al-R-), lactating (primiparous) receptive does (Al+ R+) and lactating (primiparous) non receptive does (Al+R-). Each

ejaculate was distributed at each class (physiological status) of does of the 2 groups. The straws were thawed in a 37°C water bath for 40 sec, carefully dried, cut at one extremity, placed in a syringe for 0.5 ml straw (I.M.V.) and the semen was immediately inseminated. Ovulation was induced by an intra-muscular injection of 0.2 ml of Receptal (DISTRIVET).
The does were fed *ad libitum* with a commercial diet containing 16.5% of protein and 15.5% of fibre and supplemented with an anticoccidial agent. Water was provided *ad libitum*.

Measurements of semen
Immediately after collection, pH, volume, general motility, individual motility, percentage of live spermatozoa, concentration (number of spermatozoa/ml estimated by hemocytometer counting) were recorded (BOUSSIT, 1989). A straw of each semen sample was thawed to register individual motility and the percentage of live spermatozoa ; the number of alive inseminated spermatozoa has been computed. All the frozen semen were kept and inseminated, whatever the individual motility was at thawing.
Reproduction performances were registered ; fertility (littering does having a total young born > 0 / inseminated does) total born, born alive and still-born, litter size, weight of the litter and mean weight of the young rabbits at weaning.

Statistical analysis
Fertility was considered as a variable of BERNOULLI (variable 0-1). For fertility, analysis of variance has taken into account the fixed effects for the ejaculate (33 levels), of the receptivity (receptives or no receptives), of the parity (nulliparous or primiparous). For all others traits, because of the poor performances of non receptive does, analysis of variance was performed only on receptive ones with fixed effects for male (12 levels) and parity (nulliparous or primiparous). Results presented in tables are least square means with standard deviation put into brackets. The calculated probability (P) is indicated "NS" when $P > 0.05$, * when $P<0.05$, ** when $P < 0.01$ and *** when $P < 0.001$. Pearson correlations was applied to study the relationships between biological parameters before and after deep freezing and the connections between them and doe performance.

RESULTS

When the does were non receptive and non lactating, in one case (male 907216), none of the six inseminated does littered (Table 1). On 66 non receptive and lactating does, only 4 of them littered. So, it was impossible to analyse the parity effect on non receptive does. Furthermore, on receptive and lactating does, the insemination of 3 ejaculates was not followed by littering. Consequently, the prolificacy and weaning performance were analysed taking into account the effects of the male and the parity of the does.
One ejaculate has been divided into 11 used straws at least. A mean dose of insemination contained 26 10^6 spermatozoa, 9 10^6 were alived after thawing (minimum = 1.5, maximum = 22 10^6 spermatozoa). At collection, 81 % of cells were alive (minimum = 55, maximum = 95%), after freezing, only 35% were motile (minimum = 9, maximum = 65%).

Table 1 : Straws distribution in relationship with the males and the physiological status of the does

			Number of inseminated does				Number of litters			
Male	Ejaculates	Straws	$L\text{-}R^+$	$L\text{-}R^-$	$L+R^+$	$L+R^-$	$L\text{-}R^+$	$L\text{-}R^-$	$L+R^+$	$L+R^-$
907209	4	69	34	16	10	9	30	9	7	0
907212	3	47	26	8	5	8	23	4	4	0
907213	1	15	9	2	2	2	9	1	1	1
907216	3	36	15	6	10	5	14	0	7	0
907217	4	51	31	7	5	8	28	2	4	0
907505	3	67	35	18	9	5	32	10	7	1
907506	4	52	29	9	7	7	26	4	4	0
907508	2	27	13	5	7	2	11	2	6	0
907510	3	65	39	11	6	9	32	3	3	0
907511	3	45	20	11	9	5	18	4	6	0
907515	1	23	15	3	2	3	15	1	2	2
907516	2	28	12	7	6	3	12	3	3	0

L^- : non lactant (nulliparous) does, L^+ : lactant (primiparous) does, R^+ : receptive does, R^- : non receptive does.

Fertility

From 525 inseminated does with deep frozen semen, 66.5 % of them littered. The ejaculate does not influence (Table 2) the fertility (minimum = 45.5, maximum = 83.3 %). We verified that no male effect occurs too, on fertility. Receptive does were more fertile than non receptive ones (79.0 *vs* 20.3 %, P = 0.0001). Fertility is higher for nulliparous than for primiparous does (63.6 *vs* 35.7%, P = 0.001). Receptivity and parity did not interact significantly, but only 3.2% of primiparous non receptive does littered.

Table 2 : Analysis of variance results. Ejaculate effect, does receptivity and parity effects on fertility

	N	Fertility (%)
Ejaculate		NS
Receptivity		***
Receptives	*356*	*79.0 (2.30)*
Non receptives	*169*	*20.3 (3.13)*
Parity		***
Nulliparous	*381*	*63.6 (2.30)*
Primiparous	*144*	*35.7 (3.25)*
R^2		0.52

Prolificacy

At birth (Table 3), the litter size averaged 9.1 young rabbits and 8.4 of them were alive. The male influences the prolificacy of does. At birth, the total number of pups varied from 5.6 to 11.5 and those born alive from 5.2 to 10.9. Primiparous does had higher litter size than nulliparous (10.6 *vs* 8.9 total born, P = 0.001 and 9.6 *vs* 8.1 born alive, P = 0.004). The male and the parity do not interact.

Table 3 : Analysis of variance results. Male and does parity effects on prolificacy and weaning performances. Receptive does only.

	N	Total born /litter	Born alive /litter	Still-born /litter	*N*	Weaned /litter	*N*	Mean weight at weaning
Male		***	***	*		*		***
Parity		***	***	NS		***		NS
Nulliparous	*250*	*8.89 (0.17)*	*8.13 (0.21)*	0.76 *(0.13)*	*228*	*7.54 (0.17)*	*224*	590 *(8.1)*
Primiparous	*54*	*10.63 (0.40)*	*9.64 (0.48)*	0.99 *(0.31)*	*50*	*9.03 (0.40)*	*50*	622 *(18.4)*
R^2		0.30	0.23	0.10		0.25		0.27

Weaning performances

At weaning (Table 3), the litter size averaged 7.9 young rabbits, they weighed 587g. The male influences the litter size at weaning (P = 0.046) and the mean weight of the young (P = 0.0009). Twenty eight days *post partum*, the number of young varied from 5.3 to 9.8 and their mean weight from 493 to 723g. The effect of the parity of the does at insemination, on prolificacy at birth, was maintained until weaning (respectively 9.0 *vs* 7.5 weaned young for primiparous and nulliparous, P = 0.0007), without effect on the mean weight. The effect of the male and the parity of the does did not interact on weaning performance.

Correlations between biological parameters of semen and does reproductive performance

The individual motility and the percentage of motile cells has been measured before and after freezing. There were not significant correlations on individual motility and on the percentage of motile cells before freezing and after thawing. Furthermore, none of the biological parameters of the semen were significantly correlated with the fertility, the prolificacy of the does or weaning performance of the young rabbits. The most important correlation with fertility was with the volume (r = 0.33, figure 1a), and with the number of born alive, the percentage of motile cells after thawing (r = 0.22, figure 1b).

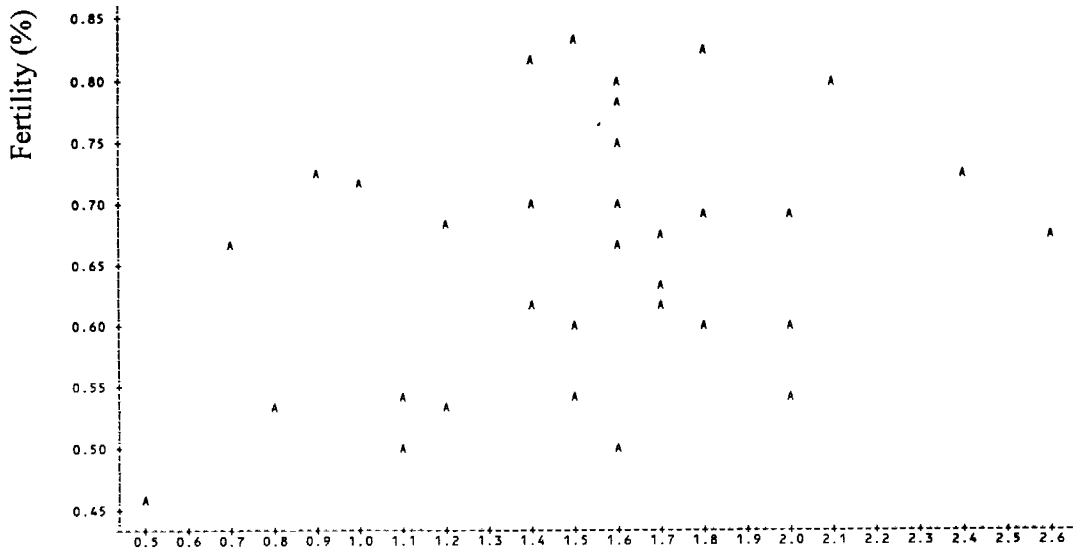

Figure 1a

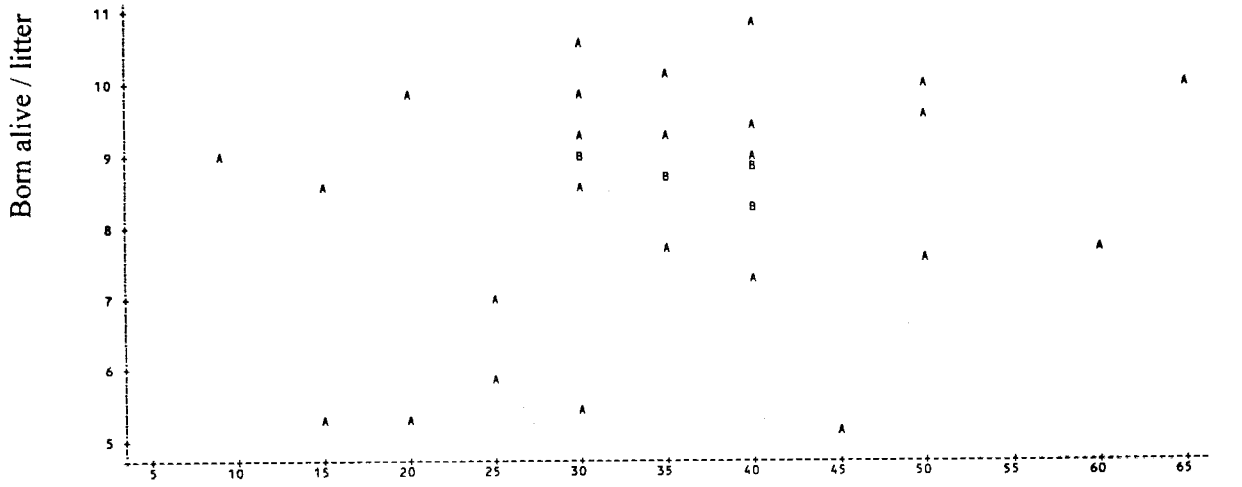

Figure 1b

Figure 1 Relationships between semen biological parameters and does reproductive performances.

Legend : A = 1 observation, B = 2 observations ....

DISCUSSION

Generally, experiments concerning the comparison between fresh and deep frozen semen, show that freezing significantly affects fertility and prolificacy (BATTAGLINI *et al.*, 1981 ; FARGEAS, 1995). Nevertheless, some authors do not agree but generally register reproductive performances on a quite low size sample (GRASER, 1978 ; CHEN *et al.*, 1990).

Fertility in our study is slightly lower than those of ANDRIEU and COUROT (1976, 84 *vs* 66%), but litter size at birth is higher (6.9 *vs* 9.1). The number of motile inseminated cells greatly vary between authors : from 1.6 (CHEN *et al.*, 1989 ; CHEN *et al.*, 1990) to 7 to 26 10^6 (ANDRIEU and COUROT, 1976). In our experiment, the minimum and the amplitude were higher (9 to 65 10^6) and can perhaps explain the lack of significant correlations between the fertility or the prolificacy and the number of inseminated cells alive. Consequently, this suggests that decreasing the number of inseminated cells is possible without affecting the reproductive performance of the does, but further studies are necessary to precise the optimal number.

Even if control inseminations with fresh semen was not performed in our experiment, the results obtained suggest that 5 years storage does not affect the fertility ability of the semen as concluded by MAURER *et al.* (1976), GRASER (1978) and WEITZE *et al.* (1982) on shorter periods (12 to 15 months). Nevertheless, freezing causes cellular damage respectively before and during freezing or during thawing (COURTENS and THEAU-CLEMENT, 1996) leading as suggested by MAURER *et al* (1976), to fertilization failures and / or important embryo losses before implantation. Effectively, after "*in vitro*" culture, these authors showed that embryos coming from frozen semen were smaller than others.

We do not find any ejaculate effect on fertility suggesting that the semen characteristics do not influence the fertilization process. Nevertheless, the males influence the prolificacy and the weaning performance of the does. CHEN *et al.* (1989) concluded that the male significantly affected pregnancy rate and litter size and suggested that there exits differences in sperm survival among them.

In agreement with THEAU-CLEMENT and ROUSTAN (1992) after fresh semen inseminations, THEAU and ROUSTAN (1982) and CASTELLINI *et al.* (1988) with frozen semen, receptive does at insemination time are more fertile than non receptive ones.

As shown by POUJARDIEU and THEAU-CLEMENT (1995) on natural mating (only receptive does), parity of the does greatly influences the fertility and the litter sizes at birth and at weaning. The nulliparous are more fertile but the primiparous does had higher litter sizes, but because of PMSG injection (prior primiparous insemination) we cannot dissociate the parity effect from the lactating effect and PMSG effect. Furthermore, the primiparous does were inseminated in summer, half of the nulliparous does in summer and winter for the others.

We did not find any significant correlations between biological parameters of the semen before freezing and after thawing. PINATEL *et al.* (1980) concluded that the residual motility of human spermatozoa after thawing is not a valuable indication of their fertilising power. This quite disappointing observation suggests that usual visual parameters are ineffective in predicting the ability of semen to be frozen and similarly could explain the failure of significant correlations with reproduction performance of the does. New objective measurements are necessary to detect semen with high freezing aptitudes in order to improve frozen semen results.

CONCLUSION

In light of the results, we are able to conclude that, in our experimental conditions, the fertility is largely affected by the lack of receptivity of the does, more than by poor biological characteristics of the deep frozen semen. On a smaller scale, parity influences fertility and prolificacy. Nevertheless, the male seems to influence the prolificacy as well as the weaning performance of the young. In conclusion, on receptive does, deep frozen semen, preserved five years in liquid nitrogen, can lead to good reproduction performance.

Acknowledgements - The authors are gratefully to B. POUJARDIEU for critically reading the manuscript, as well as for helpful advice during the course of the work. They thank Véronique TARTIE who was in charge of semen cryopreservation, we greatly appreciated her collaboration, all the staff of the SELAP and experimental farm of Le Magneraud. We thank particularly J. ESPARBIE, Y. ANDREUZZA and Jacqueline RUIZ for their technical assistance.

REFERENCES

ANDRIEU R., COUROT M., 1976. Congélation du sperme de lapin en vue de l'insémination artificielle. *Ier Congrès Mondial Cunicole, 31 Mars-2 Avril, 1976, Dijon, France,* **Comm.67**, 4 pp.

BATTAGLINI M., COSTANTINI F., CASTELLINI C., 1988. Fecondazione artificiale del coniglio con sperma refrigato e congelato. *Zootecnica e nutrizione animale*, **3**, 267-272.

BOUSSIT D., 1989. *Reproduction et insémination artificielle en cuniculture.* Association Française de Cuniculture, Lempdes, Paris. pp. 234.

CASTELLINI C., COSTANTINI F., BATTAGLINI M., 1988. Fecondazione artificiale del coniglio. *Rivista di Coniglicoltura*, **7**, 45-47.

CHEN Y.Q., LI J., SIMKI M.E., YANG X., FOOTE R.H., 1989. Fertility of fresh and frozen rabbit inseminated at different times as indicative of male differences in capacitation time. *Biology of reproduction*, **41(5)**, 848-853.

CHEN Y.Q., BLANPAIN-TOBBACK LI J.M., YANG X., FOOTE R.H., 1990. Improvements in freezing rabbit spermatozoa for biotechnology studies. *Journal of Reproduction and Fertility Suppl.*, **41**, 217.

COURTENS J.L., THEAU-CLEMENT M., 1996. Ultrastructural modifications of rabbit spermatozoa induced by two freezing and thawing techniques. *VI th Congress of World Rabbit Science Association, July 9-12, 1996, Toulouse*, France., (submitted for publication).

FARGEAS E., 1995. Quelques résultats obtenus en insémination artificielle avec de la semence congelée. *Cuniculture*, **22(3)**, 103-106.

GRASER A., 1978. Effect of long-term storage of deep-frozen rabbit semen on sperm characteristics and fertilising ability. *Thesis, Tierärztliche Hochschule.* pp. 69.

MAURER R.R., STRANZINGER G.F., PAUFLER S.K., 1976 Embryonic development in rabbits after insemination with spermatozoa stored at 37.5 or -196°C for various periods. *Journal of Reproduction and Fertility*, **48(1)**, 43-49.

PINATEL M.C., CZYBA J.F., GUERIN J.F., 1980. Lesions in human spermatozoa after preservation in liquid nitrogen. *Acta Europea Fertilitatis*, **11(4)**, 281-284.

POUJARDIEU B., THEAU-CLEMENT M., 1995. Productivité de la lapine et état physiologique. *Annales de Zootechnie* **44**, 29-39.

THEAU M., ROUSTAN A., 1982. Etude des possibilités de dilution du sperme de lapin congelé pour l'insémination artificielle. *3èmes Journées de la Recherche Cunicole, 8 et 9 décembre, 1982, Paris.* **Comm.19.**

THEAU-CLEMENT M., POUJARDIEU B., BELLEREAUD J., 1990. Influence des traitements lumineux, modes de reproduction et états physiologiques sur la productivité de lapines multipares. *5èmes Journées de la Recherche Cunicole, 12-13 Décembre, 1990, Paris*, **Tome I**, Comm.7.

THEAU-CLEMENT M,. ROUSTAN A., 1992. A study on relationships between receptivity and lactation in the doe, and their influence on reproductive performances. *Vth Congress of World Rabbit Science Association, July 25-30, 1992, Corvallis, U.S.A.*, **Vol A**, 412-421.

WEITZE VON K.F., SCHARNHÖLZ A., BADER H., 1982. Tiefgefrierkonservierung von Kaninchensperma II. Einfluß der Langzeitlagerung auf die Befruchtungsfähigkeit. *Zuchthygiene* **17**, 172-177.

Performances de reproduction de lapines inséminées avec de la semence congelée - Dans cette expérience, les résultats d'insémination artificielle de 381 lapines nullipares (non allaitantes) et 42 jours après, de 144 lapines primipares (allaitantes) ont été analysés. Les lapines étaient inséminées avec de la semence congelée et stockée durant 5 ans dans de l'azote liquide. 525 paillettes issues de 33 éjaculats provenant de 12 males ont été utilisées. Les paillettes de chaque éjaculat ont été équitablement réparties en fonction de la saison, de la réceptivité sexuelle des lapines et de leur stade physiologique. La fertilité, en moyenne de 66%, est influencée par la réceptivité (réceptives : 79%, non réceptives : 20%) et le stade physiologique des lapines (non allaitantes : 64%, allaitantes : 36%). A cause de leur faible fertilité, les lapines non réceptives n'ont pas été intégrées dans l'analyse de la productivité numérique et pondérale. Les tailles de portée à la naissance (9.1 nés totaux, 8.4 nés vivants) et au sevrage (7.9 sevrés) sont influencées par le mâle, père de la portée et le stade physiologique des lapines. Le mâle seul, influence le poids moyen des lapereaux au sevrage. La motilité individuelle et le pourcentage de cellules mobiles ont été mesurés avant congélation et après décongélation ; aucune relation n'est observée entre ces observations. De plus, aucune relation n'est mise en évidence entre les paramètres biologiques de la semence et les performances de reproduction des lapines.
En conclusion, il est à nouveau démontré l'effet majeur de la réceptivité sexuelle des lapines (au moment de l'insémination) sur leurs performances de reproduction. Sur des femelles réceptives, l'insémination de semence préalablement congelée et conservée 5 ans, donne de bonnes performances de reproduction.