

## Effect of SpermVital® technology on conception rate in repeat breeder multiparous dairy cows: preliminary results

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**Abstract:** The aim of this study was to investigate the effect of SV technology on conception rates in repeat breeder multiparous dairy cows. Seventy-nine multiparous Holstein cows from a private dairy farm were used in the study. These animals were cows that had failed to conceive from at least 3 regularly spaced services (repeat breeders). Estrus cycles of the cows were synchronized by 2 injections of the PG analogue, administered 11 days apart. GnRH was applied 48 h after the second injection of PG. Twenty-four h after this administration, the animals were randomly divided into 2 groups, control and SV. The animals in the control group (n = 28) were inseminated with standard processed semen, and the cows in the SV group (n = 51) were inseminated with SV technology processed semen. A lower pregnancy rate (35.5%) was determined in the control group than in the SV (47.1%) group. The difference between pregnancy rates in the groups was statistically significant (P < 0.05). We are at too early a stage to say that SV Technology can fully respond to the deficiencies in herd management. This work may also lead to future studies into the use of more animal material.

**Key words:** Artificial insemination, repeat breeder, SpermVital® technology

### 1. Introduction

Nowadays, modern industrial milk cows are subfertile. Many researchers accept the presence of antagonism between the amount of milk produced and fertility (1,2). Pregnancy losses in dairy cows are still considered to be unresolved problems among the causes of infertility. Many investigators have linked the main cause of pregnancy losses to early or late embryonic mortality after fertilization (3,4). Indeed, embryonic deaths represent a serious loss for breeders, at rates of 6.3%–42.7% (5,6). Embryonic deaths can be responsible for pregnancy losses after fertilization; however, clear information about failures before fertilization is rather limited. According to researchers on the reproduction of milk cows, the fertilization rate is between 90%–100% (4,7), but the perfect functioning of the complex ovulation mechanism in the success of fertilization alone is not considered sufficient. Fertilization also requires error-free coordination of the oocyte's and sperm's oviduct contact time. Failure to achieve this timing before fertilization can be shown to be a reason for pregnancy losses, but there is very little information in this regard.

The importance of ovulation and artificial insemination timing is further highlighted by the fact that in beef cattle,

fertilization failures are higher than embryonic mortalities (8). Ovulation occurs in dairy cows approximately 10–12 h after the end of estrus. In practice, this is taken into account in the artificial insemination (AI) timing. However, despite the traditional AI practice, the initial start time of estrus is not known exactly. Even if estrus time is known, the time of ovulation can vary between individual cows. In the case of delayed ovulation, AI may fail even if done during estrus. On the other hand, 5%–30% of AI is not performed during estrus, depending on the expertise of the insemination management (9). For this reason, the perfect detection of estrus is the most important condition for determining the correct insemination time (10). The optimal time of AI is related to the lifespan of the gametes. The ability of sperm to fertilize is about 24 h in natural mating, and about 18–20 h for frozen semen (11). Can the prolongation of longevity of spermatozoa in the female genital system increase the chance of fertilization? In response to this hypothesis, a new semen preparation technology, SpermVital® (SV), has been proposed. The SpermVital® technology is a Norwegian product that promises to double the life of sperm in the cow after insemination. SV embeds the semen in a matrix (alginate gel) which slowly dissolves in

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the cow and releases the semen (12,13). As a result, the correct timing of insemination in relation to ovulation will become a less crucial factor, and the chances of conception should increase. The technology has a market share of 20% in Norway. It is patented worldwide, and is now in use in most European countries.

A very limited number of SV technology studies have been performed in normally fertile cows, and the positive success has provoked excitement (12,13). Repeat breeding is one of the major infertility problems of the dairy cattle industry (14). The effect of SV technology on pregnancy rates in repeat breeder (RB) dairy cows is unknown. For this reason, the aim of this study was to investigate the effect of SV technology on the conception rates in repeat-breeder multiparous dairy cows. The present study is the first for the use of SV in repeat-breeder cows, and it can be the basis for future studies.

## 2. Materials and methods

### 2.1. Animals

Seventy-nine multiparous Holstein cows from a private dairy farm (Sivas, Turkey, 39°51'28.9"N, 36°20'17.5"E) were used in the study. The limitation of animal material to 79 stemmed from the fact that businesses do not keep infertile animals at their farms for a long time. The cows were in their third to seventh lactation and were managed under similar conditions, with an average daily milk yield of 24 kg per cow. These animals were cows that had failed to conceive from at least 3 regularly spaced services (repeat breeder), and the time elapsed from the last birth was 8 months.

### 2.2. Methods

Estrous cycles of the cows were synchronized by 2 injections of a PGF<sub>2α</sub> (500 µg D-Cloprostenol, Estrumate<sup>®</sup>), administered 11 days apart from each other. GnRH (10 µg Buserelin, Receptal<sup>®</sup>) was applied 48 h after the second injection of PGF<sub>2α</sub>. After this administration, the animals were randomly divided into 2 groups, control and SV. The animals in the control group (n = 28) were inseminated twice with standard semen 72–96 h after the second injection of PGF<sub>2α</sub>. The cows in the SV group (n = 51) were inseminated only once, at 72 h. The semen used for insemination of the animals in the control group were examined before the study by phase-contrast microscopy (Axioscope A1, Zeiss, Germany). As a result of these examinations, sperm with a motility rate of at least 60% and concentration of  $15 \times 10^6$  per straw were included in the study. In the control group, the same batch of sperm from a Holstein bull was used. The sperm used in the SpermVital<sup>®</sup> group was examined only at the import stage and within the ministry, as special techniques were required. According to the information obtained from the

manufacturer, the sperm we used for the SV group had a motility rate of at least 50% and a concentration of 12 million sperm/straw. The AIs were always performed by the same person. Pregnancy examinations were performed by transrectal ultrasonography on days 32 and 60 after AI.

### 2.3. Statistical analysis

The chi-square test was used to assess differences in pregnancy rates among the groups. Statistical analyses were performed with a minimum error margin of 5%. The SPSS 14.01 package program was used.

### 2.4. Ethical approval

There was no need for approval from the ethics committee during the process.

## 3. Results

Date of pregnancy rates following PG–GnRH synchronization and AI protocols in the groups are presented in Table. In the control group, a lower pregnancy rate (35.5%) was determined than in the SV (47.1%) group. The difference between pregnancy rates in the groups was statistically significant ( $P < 0.05$ ). Numerically, this result showed that SV technology could provide more economic gain than standard sperm by increasing pregnancy rates in repeat breeder cows (Table).

## 4. Discussion

SV Technology is based on the principle of increasing the lifespan of sperm cells in the cow genital tract (up to 48 h) (15). Increasing fertility by eliminating faults in insemination and ovulation is the main economic advantage expected from SV Technology. However, neither in vitro nor in vivo studies have yet fully elucidated the effects of SV Technology on postinsemination fertility. In a study conducted in both in vivo and in vitro conditions (13), it has been reported that SV Technology should be further investigated. In this study, 7081 conventional inseminations were compared with 7044 inseminations prepared with SV technology. The fertility success after both inseminations was based on the “56th day nonreturn ratio (NRR)”. According to this fertility parameter, SV technology was not superior

**Table.** Effect of AIs with different processed semen (standard or SV<sup>®</sup> technology) on pregnancy rates in the control and SV groups.

Groups	N	Pregnancy rate % (n)
Control	28	35.7 <sup>a</sup> (10)
SV	51	47.1 <sup>b</sup> (24)

Pearson chi-square: 0.330 ( $P < 0.05$ ).

to standard insemination (control: 72.5%, SV: 72.7%). Furthermore, in their field study, the inseminations were carried out by different veterinarians. Insemination by different persons can have an effect on NRR values in both groups. In addition, pregnancy examination was not performed in this study with rectal or ultrasound examinations, but based on the fact that these animals showed estrus. The absence of signs of estrus is not a sufficient criterion for positive pregnancy. But the *in vitro* flow cytometric findings of the same study reveal even more interesting results regarding SV technology. According to plasma membrane and acrosome integrity tests, SV technology causes degeneration worse than standard method extenders in sperm cells. These results have led to a contradictory question: why can SV technology achieve the same fertility success with this technique in field conditions, even if it causes significant degeneration in sperm cells? The answer to this question can be explained by the fact that SV survival is 2 times higher than standard semen survival. A study by Alm-Kristiansen et al. (12) found striking results. In this new study, according to *in vitro* observations, it was expressed that the survival rate of 48 h after thawing of SV sperm cells was higher than that of standard sperm cells. The fact that SV technology does not require a second dose of insemination can be considered a notable advantage of this technology (12). However, even if SV technology is preferred to double insemination, the rate of pregnancies that can be achieved with cows in this way still remained below 50% (12). The pregnancy rate of 47.4% in cows cannot be regarded as an important success criterion for SV technology. However, they found that the chance of pregnancy in heifers using SV technology is higher than in cows. In this case, it would be more practical and economical to choose SV instead of double-dose insemination in heifers. A recently published study (16) has been limited to *in vitro* observations in sperm cells produced by SV technology. However, this study has provided the development of a second-generation SV system. The comparative results of this study showed that sperm viability and acrosome integrity after thawing were higher in second-generation SV sperm cells than in first-generation SV and standard (Biladyl) sperm cells ( $P < 0.05$ ). The presence of differences in DNA quality in all sperm cells (second-generation and first-generation SV and standard sperm cells) was also noted in the study results. However, the important negative effects of sperm DNA damage on reproduction cannot be debated (17). In this case, the results of *in vivo* field conditions of sperm cells (16) produced with second-generation SV technology could more accurately reflect the significance of this work. In our study, we could achieve a pregnancy rate of 47% with SV technology in the repeat-breeder

multiparous cows. This pregnancy ratio was significantly higher than in the control group (35%) ( $P < 0.05$ ); SV technology seems to be more successful in terms of pregnancy rate than the control. Linking the cause of the 12% difference in pregnancy rate to SV technology alone will not be accepted as an objective evaluation. Alm-Kristiansen et al. (12) could achieve a pregnancy rate of only 47.4% in healthy cows with SV without ovulation stimulation. In our study, ovulation was induced by GnRH injection 24 h before SV insemination. The effect of GnRH injection on ovulation is related to the dominant follicle diameter (18). Synchronization programs can also influence pregnancy rates (19). However, considering the 12% difference in our study, SV technology can only be used in cows that breed again because of insufficient insemination timing. It should not be forgotten that SV technology alone may be inadequate against other factors in repeat breeding. Alm-Kristiansen et al. (12) showed a 16% increase in pregnancies achieved with SV between heifers and cows, suggesting that uterine conditions also influence the SV results. It would be unrealistic to expect success from SV alone in milk cow management. As a management factor in addition to performance-adapted feeding (20,21) and good health status (22), reliable heat detection and timely insemination with high-quality semen play important roles (23).

Consequently,

- Pregnancy success cannot be completely attributed to quality and life span of sperm. Pregnancy success is directly related to ovulation time and uterine environment, as well as oocyte and embryo quality.

- Our study shows that SV technology can increase pregnancy rates in repeat-breeder cows.

Our research showed that SV technology can minimize infertility problems due to incorrect and early insemination.

- Up to the present, the maximum pregnancy rate in cows with SV has been less than 50% (47.4%). This rate has increased to 63.5% with an increase of about 16% in heifers (12). This dramatic difference in pregnancy rates between cows and heifers emphasized the necessity to take into consideration for pregnancy rates factors other than uterine and embryo-related factors, especially during sperm life.

- We are at too early a stage to say that SV technology can fully respond to the deficiencies in herd management. The efficacy and *in vitro* fertilization performance of SV technology should be proven by further studies in this field. This work may also lead to future studies into the use of more animal material.

#### **Conflict of interest**

The authors declare that they have no competing interests.

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